Human Experimental Endotoxemia in Modeling the Pathophysiology, Genomics, and Therapeutics of Innate Immunity in Complex Cardiometabolic Diseases

Parth N. Patel, Rhia Y. Shah, Jane F. Ferguson, Muredach P. Reilly

Abstract—Inflammation is a fundamental feature of several complex cardiometabolic diseases. Indeed, obesity, insulin resistance, metabolic dyslipidemia, and atherosclerosis are all closely linked inflammatory states. Increasing evidence suggests that the infectious, biome-related, or endogenous activation of the innate immune system may contribute to the development of metabolic syndrome and cardiovascular disease. Here, we describe the human experimental endotoxemia model for the specific study of innate immunity in understanding further the pathogenesis of cardiometabolic disease. In a controlled, experimental setting, administration of an intravenous bolus of purified Escherichia coli endotoxin activates innate immunity in healthy human volunteers. During endotoxemia, changes emerge in glucose metabolism, lipoprotein composition, and lipoprotein functions that closely resemble those observed chronically in inflammatory cardiovascular disease risk states. In this review, we describe the transient systemic inflammation and specific metabolic consequences that develop during human endotoxemia. Such a model provides a controlled induction of systemic inflammation, eliminates confounding, undermines reverse causation, and possesses unique potential as a starting point for genomic screening and testing of novel therapeutics for treatment of the inflammatory underpinning of cardiometabolic disease.

(Revised August 24, 2014; final version accepted December 18, 2014.)

Key Words: cytokines, chemotactic ■ immune system ■ inflammation ■ metabolic syndrome X

Innate Immunity and Cardiometabolic Disease

Innate immunity, an ancient form of host defense, is the body’s rapid, first-line response to environmental threats, such as microbial infection. In contrast to the adaptive immune system—which is present only in higher-order vertebrates, and mediated primarily by somatically generated receptors—the innate immune system relies inherently on basic detection machineries coded for and conserved within the germ-lines of higher and lower organisms, from plants and fruits flies to mammals. For the specificity of innate immune receptors to be conferred genetically, innate immune recognition must be built on small families of membrane receptors that recognize highly conserved pattern structures present in large groups of microorganisms. Perhaps the most prominent and widely studied subgroup of these pattern recognition receptors is the toll-like receptor (TLR) family, whose 10 members are manifest in humans as cell surface receptors in a series of trouble-detecting sentinel cells. Individual TLRs are known to play important roles in the recognition of structures derived from pathogens, such as fungi, protozoa, viruses, and bacteria. As such, the TLR family is now widely accepted as the major microbe sensing system in mammals.

A classic starting point for innate immunity is TLR-4, which—through detection of bacterial lipopolysaccharide—is crucial for the effective immune response to Gram-negative bacteria. The binding of lipopolysaccharide to TLR-4 leads to downstream activation of nuclear factor-xB (NF-xB), a nuclear transcription factor responsible for regulating gene products that initiate a generalized inflammatory response. Specifically, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and other proinflammatory mediators are all synthesized and released into the systemic circulation, where they trigger the activation of the complement system, the coagulation cascade, and the acute phase response. TNF-α and IL-1β also play key roles in facilitating leukocyte migration by increasing the expression of adhesion molecules on endothelial cells. Finally, the presence of circulating inflammatory cytokines enhances tissue perfusion, vascular permeability, and cell migration throughout the body. Altogether, these systemic changes work together to allow for the timely and efficient eradication of the invading microorganism.

Inappropriate or sustained triggering of innate immunity and inflammatory signaling may, however, contribute to various medical conditions and diseases. Excessive activation of inflammatory cytokines leads to septic shock, a leading cause...
of death in patients with bacterial infections.\textsuperscript{11} Several studies indicate that more moderate TLR-4 activation is also linked to immunodeficiency, asthma, obesity, diabetes mellitus, and atherosclerosis,\textsuperscript{12–15} all of which are known to possess substantial inflammatory components. An inflammatory insulin resistance (IR) and metabolic dyslipidemia emerges clinically during acute sepsis\textsuperscript{16} and chronic infections,\textsuperscript{17} possibly via activation of TLR-4 signaling. Furthermore, experimental studies of TLR-4 deficiency in mouse models demonstrate a reduction in both diet-induced obesity\textsuperscript{18} and atherosclerosis.\textsuperscript{19} Finally, genetic manipulation and therapeutic targeting of TLR-4\textsuperscript{20} and NF-κB\textsuperscript{21,22} have provided proof of concept that modulation of innate immune signaling attenuates IR and type 2 diabetes mellitus in dietary and obesity models. Taken together, therefore, several lines of evidence suggest that chronic TLR-4 activation by exogenous and host-derived molecules may lead to a pro-inflammatory state of increased cytokines, chemokines, and adhesion molecules, all of which can exacerbate the risk of cardiometabolic disease.\textsuperscript{23}

**Human Experimental Endotoxemia: An Introduction and History**

Human experimental endotoxemia has emerged as a controlled model for the study of complex disease inflammatory responses and their modulation in vivo. Administration of an intravenous bolus of purified *Escherichia coli* endotoxin activates TLR-4 signaling and stimulates innate immunity in healthy human volunteers.\textsuperscript{24} Although administration of lower doses of *E coli* lipopolysaccharide (0.2–2 ng/kg body weight) is best acknowledged today as a transient model of moderate systemic inflammation, intravenous lipopolysaccharide for decades was used at higher doses (3–5 ng/kg body weight) to mimic the storm of inflammatory signaling seen in acute clinical inflammatory conditions, such as bacteremia and sepsis.\textsuperscript{25–28} Indeed, sepsis-like changes in systemic hemodynamics, ventricular function, pulmonary gas exchange, and permeability have all been shown to emerge within hours of experimental administration of higher doses of lipopolysaccharide.\textsuperscript{29} These responses are driven by a sharp induction of proinflammatory cytokines (eg, TNF-α, IL-1β, IL-6, and IL-8), many of which circulate in the plasma at levels resembling those seen clinically in infection and early sepsis.\textsuperscript{29} Because the pathophysiological derangements observed in septic patients result from an acute, systemic inflammatory response to endotoxin derived from Gram-negative bacteria, it was logical to first use experimental endotoxemia most directly, and proximally, as a controlled model for the study of sepsis.

As such a controlled, transient model of early sepsis, human experimental endotoxemia also offered a means to study multiple organ dysfunction in septic shock, a topic intrinsically difficult to investigate in critically ill patients. Using thermomodulation pulmonary-artery catheters and simultaneous radionuclide cineangiography, Suffredini et al\textsuperscript{24} were able to monitor the initial cardiovascular effects of 4 ng/kg endotoxin in healthy volunteers. Experimental endotoxemia resulted in a hyperdynamic cardiovascular state involving an early increase in cardiac index with a concurrent reduction in systemic vascular resistance. An elevated heart rate and reduced mean arterial pressure were also manifested, altogether suggesting that experimental endotoxemia qualitatively mimicked the hyperdynamic circulatory pattern observed in septic shock. During endotoxemia, left ventricular ejection fraction was significantly depressed, whereas end-diastolic and end-systolic volume indexes both increased. Decreased myocardial contractility was further evidenced by a reduced ratio of peak systolic pressure:end-systolic volume index, an observation consistent with clinical studies of septic shock.\textsuperscript{30–33} Indeed, the presence of diminishing left ventricular function in manners analogous to clinical septic shock demonstrated that endotoxin, its detection machineries, and its signaling mechanisms also possessed biological relevance to sepsis-related cardiac dysfunction in humans.

**Human Experimental Endotoxemia as a Model for Cardiometabolic Disease**

As a growing collection of recent literature has investigated the intricacies of the endotoxemia model, it has become widely acknowledged that human experimental endotoxemia may actually not be best-defined as a model of sepsis, but rather one of moderate systemic inflammation.\textsuperscript{9,34,35} Particularly, at lower doses of lipopolysaccharide, endotoxemia activates innate immunity at a level vastly more relevant to the low-grade, chronic inflammatory state observed in cardiometabolic disease.\textsuperscript{36,37} In contrast to the near-supraphysiologic hundred to thousand-fold increases in TNF-α after higher doses of lipopolysaccharide,\textsuperscript{38,39} administration of lower doses leads to modest, several-fold increases in plasma levels of cytokines,\textsuperscript{40,41} which more closely reflects, albeit in an acute manner, the subclinical inflammation, which characterizes the metabolic syndrome and chronic cardiovascular diseases (CVDs).\textsuperscript{42–44} Moreover, as a model for the inflammatory contributions to cardiometabolic disease, experimental endotoxemia has strong biological plausibility because it activates pathways known to be perturbed in obesity, diabetes mellitus, and atherosclerosis.\textsuperscript{18–20} Indeed in settings of risk for cardiometabolic disease, TLR-4 is activated intermittently, both locally and systemically, by host-derived antigens that are generated and circulated at more modest concentrations and thereby establish a dynamic low-grade inflammatory state even in sterile, noninfectious settings. Thus, at lower doses, *E coli* endotoxemia is reasonably thought to have substantial relevance to diseases associated with subclinical activation of innate immunity.\textsuperscript{36,45} A recent development that supports the legitimacy of the human endotoxemia model is an increasing awareness of the
gut microbiome as a dynamic inflammatory and metabolic influence in human disease. In fact, systemic and recurrent episodes of low-grade inflammation may result from metabolic endotoxemia and metabolic bacteremia, 2 phenomena in which bacterial fragments or live bacteria cross the gut mucosal membrane and enter into the systemic circulation.\textsuperscript{46,47} Mounting evidence suggests that high-fat diets increase gut permeability, resulting in 2- to 3-fold postprandial increases of bacterial lipopolysaccharide in the host circulation,\textsuperscript{48–51} while also generating, via altered gut microbiome, systemically active metabolites that directly affect cardiometabolic diseases.\textsuperscript{52,53} Although postprandial circulating levels of lipopolysaccharide are notably 10 to 50x lower than the levels observed in septicemia and infections, metabolic endotoxemia nonetheless activates TLR-4–dependent innate immunity and seems to serve as an important determinant in the pathogenesis of inflammatory-induced obesity and type 2 diabetes mellitus.\textsuperscript{46} Distinct gut microbiota signatures—likely conferred by long-term diet—have been linked with inflammatory, obese, and metabolic conditions,\textsuperscript{54–57} and modulation of gut microbiota signatures in animal models has proven to relieve metabolic dysfunction.\textsuperscript{58,59} In addition, atherosclerotic plaque contains microbes, likely oral and gut derived,\textsuperscript{60} whereas blood microbial load may be predictive of the development of diabetes mellitus.\textsuperscript{61} Our growing understanding that the immune response to bacteria is closely linked to cardiometabolic disease risk emphasizes further both the relevance and the use of human endotoxemia protocols.

**Evoked Inflammation Induces Cardiometabolic Disturbances in Humans**

After the administration of lipopolysaccharide in humans, several changes emerge that closely resemble those chronically observed in CVD risk states (Figure [A]). To begin, experimental endotoxemia leads to significant system-wide alterations in glucose homeostasis. Agwunobi et al\textsuperscript{62} were the first to document impaired insulin sensitivity after endotoxin administration in humans. More recently, our group further demonstrated that endotoxemia leads to the loss of both hepatic and peripheral insulin sensitivity.\textsuperscript{63} In a much larger sample, we have confirmed endotoxemia-induced IR in both European and African ancestry populations and have revealed an apparent compensatory increase in pancreatic \(\beta\)-cell insulin secretion and function.\textsuperscript{64} Furthermore, we have shown that endotoxemia induces substantial adipose tissue inflammation—characterized by the upregulation of chemokines, T-cell markers, macrophage markers, and many other genes—in a manner that parallels the abnormalities observed in adipose tissue in obesity and obesity-related IR.\textsuperscript{37,63,65,66} Importantly, during experimental human endotoxemia, this adipose tissue inflammation has been shown to precede systemic IR.\textsuperscript{63} Finally, our group has also shown that a subclinical, low-dose
(0.6 ng/kg) endotoxemia produces a more subtle adipose tissue inflammation and a more modest IR more consistent with the extent of abnormality observed in metabolic syndrome and diabetes mellitus.66

Inflammatory conditions are also characterized by widespread changes in plasma lipoproteins,67,68 some of which may directly exacerbate the risk of cardiometabolic complications and atherosclerosis.69,70 Hudgins et al71 showed that intravenous endotoxin in healthy human volunteers reproduces many of the lipid and lipoprotein changes observed in sepsis and atherogenic dyslipidemia (ie, an increase in plasma triglycerides, an increase in small dense low-density lipoprotein particles, high-density lipoprotein (HDL) remodeling, and a reduction in HDL particle size). Notably, these changes included a marked increase in HDL-cholesterol levels and a decline in HDL phospholipid, all while apolipoprotein A-I and HDL-cholesterol levels remained constant. More recently, our group examined the functional consequences of lipopolysaccharide-induced HDL remodeling and demonstrated that endotoxemia triggers HDL dysfunction—specifically by impairing HDL-macrophage cholesterol-efflux function, the first step in reverse cholesterol transport72—independent of changes in plasma HDL-C and ApoA-I levels.73,74 We observed that the activation of innate immunity modulates HDL composition, in particular, by inducing a substantial loss of HDL phospholipid and a specific decrease in small- to medium-sized HDL particles. Importantly, these changes followed a simultaneous induction of both HDL lipases and HDL enrichment with serum amyloid-A73–75 and coincided with an impaired capacity of the isolated HDL to efflux cholesterol from macrophages.73,74 This loss of HDL reverse cholesterol transport function is a pathological hallmark of acute and chronic recurrent clinical inflammatory syndromes that are associated with an increased risk of atherosclerosis and acute cardiovascular events. In fact, reduced HDL cholesterol-efflux function has been observed in IR,76 obesity,77 psoriasis,78 systemic lupus erythematosus,79 acute infections,80 and surgery-induced systemic inflammation80 and has been shown to be an independent risk factor for coronary artery disease independent of HDL-C levels.81 Together, these human data underscore the clinical relevance of experimental endotoxemia in the study of the atherogenic dyslipidemia found in inflammatory cardiometabolic diseases.

Advantages of the Model

A distinct advantage of the experimental endotoxemia model is that it controls the activation of innate immunity and its downstream responses in healthy human volunteers in a temporal manner. As such, the model eliminates both confounding and reverse causation—features of observational studies in which inflammatory changes may result from other risk factors and the disease itself rather than being causal. Thus, the model provides a controlled framework for assessing the downstream effect of induced inflammation in vivo. Typically, endotoxemia studies are performed in healthy human volunteer samples. Although this certainly reduces direct translation to specific diseases, there are obvious advantages for experimental control of confounding parameters that may affect inflammatory outcomes. Individual studies may vary, but in general controllable parameters (often exclusions) include age range, obesity, pregnancy/lactating status, chronic or recurrent medical disorders including CVD, diabetes mellitus, hypertension, malignancy, inflammatory and rheumatological disorders, HIV-1 infection, liver or kidney disease, tobacco use, or the use of any prescription medication or supplemental vitamins. Recent evidence suggests that race is a parameter that may affect response,64 whereas the influence of sex is still under debate.82 Emerging data have also demonstrated that lipopolysaccharide responsiveness varies with circadian rhythm83—consequently, most studies are performed at the same time of day, typically in the morning.

Furthermore, when attempting to predict the biochemical and clinical consequences of activated innate immunity in disease, studying the evoked physiology may be of much greater value than measuring the resting levels of inflammatory markers, the strategy in epidemiological studies. Unlike more static risk factors (eg, low-density lipoprotein-cholesterol), single time point measurements of basal circulating levels of inflammatory markers (eg, cytokines and acute phase proteins), that are putative biomarkers of CVD,84,85 may not necessarily reflect the physiology and pathophysiology of innate immune responses during dynamic disease processes in acute, subacute, or even chronic disease. In fact, resting levels in nonstressed settings may have limited relevance to how the host responds during acute or recurrent pathophysiological stresses, as has been demonstrated, particularly in response to nutritional challenges.86,87 Thus, the evoked response might be more clinically informative than basal levels. In this context, in our own work (the largest human endotoxemia protocol published to date, n=294),84 we have observed that (1) the lipopolysaccharide-induced cytokine responses had greater correlations with each other and with the subsequent increases in acute phase proteins than the correlations observed for the prelipopolysaccharide cytokines with baseline biomarkers or with lipopolysaccharide-induced responses, (2) opposite trends in basal versus endotoxemia-responses across race, with lower peak levels, but higher basal levels of inflammatory biomarkers in African ancestry when compared with European ancestry, and (3) a genome-wide significant locus for evoked fever has no association with basal temperature.88–91 Thus, basal levels may not capture the dynamic pathophysiology, may have limited use as markers of innate immune processes, and may also be relatively poor predictors of the evoked response and innate immune activity during inflammatory stress and in disease.

Experimental endotoxemia also provides a precise model for the study of the temporal patterns of innate immune responses in humans, from the early activation of systemic inflammation to the later resolution phase. This can offer a much more complete insight into the complex physiological, molecular, and genetic influences on the promotion and resolution of inflammation, insights that cannot be derived from single time point estimates or repeated sampling of resting levels in traditional population studies and clinical trials. Furthermore, by making repeated measurements on the same individual over time, the model also can account for interindividual variation. Coupled with the capacity to reveal biological differences in
innate immune responses that are either enhanced by or only evident after the experimental perturbation, this allows more modest sample sizes than traditional static epidemiological designs.92–95

Although animal models of experimental endotoxemia have benefits over human models in terms of cost, feasibility, and genetic manipulation, there are important differences between humans and model organisms that decrease the applicability of animal studies and highlight the advantages of the human experimental system. Many model organisms, including mouse and zebrafish, are lipopolysaccharide-tolerant relative to human,96,97 and thus may not be ideal models of human disease. A noteworthy study directly compared gene expression changes in human severe blunt trauma, human burn injury, 2 ng/kg human endotoxemia, mouse trauma, mouse burn injury, and mouse endotoxemia at a mathematically scaled down dose.98 Although this study concluded that mice make poor models for inflammatory diseases, a subsequent publication using the same data came to a different conclusion.99 The results from these conflicting analyses revealed that although different causes of acute inflammatory stresses result in highly similar genomic responses in humans, the responses in corresponding mouse models may only partially overlap with the human conditions. Although rodent models have specific use, the ongoing controversy underscores the need for caution in extrapolating rodent models to study human inflammatory diseases and emphasizes the value of human translational research models with direct relevance to human disease. Similarly, ex vivo endotoxemia models using human cells100,101 allow for high-throughput profiling; however, these models are not able to recapitulate the complexities of the multiple tissues and integration of cell types involved in the whole-organism inflammatory response.102

Finally, controlled endotoxemia is a useful model for the evaluation of genetic influences on evoked clinical inflammatory phenotypes and the cytokine responses that drive clinical pathophysiologies. Genetic variation in TLR4 is associated with differences in lipopolysaccharide responsiveness,103 whereas promoter polymorphisms in candidate genes, such as TNFα, IL10, and IL6, have all been studied with the intent of demonstrating the importance of specific genes and pathways on the induced inflammatory response.104,105 Recent studies have probed the cell-specific transcriptomic116 underpinning of the evoked response to endotoxemia, with novel data revealing the potential role of tissue-specific inflammatory modulation of noncoding RNA in inflammatory cardiometabolic disease.107 As a controlled model of proven relevance to inflammatory diseases, metabolic syndrome, and CVD, human experimental endotoxemia provides a probe for the study of therapeutic influences on inflammatory atherogenic stress, with important clinical and translational implications, as discussed in the next section. Altogether, experimental endotoxemia provides a well-characterized, reproducible, and tractable model of inflammation in which novel therapies and genomic influences can be tested for their ability to modulate evoked inflammation and its specific metabolic consequences. Overall, such natural genomic variations and experimental interventions offer a starting point and screening strategy for the development of novel therapies for the treatment of acute and chronic human inflammatory and cardiometabolic diseases.

Evidence for Translation and Clinical Relevance

Although the model is unable to capture the chronicity of inflammation, findings from human endotoxemia have proven to be relevant to the clinical course of both acute inflammatory and chronic inflammatory disease states. TNFα blockers and IL-1 pathway antagonists that showed partial suppression of the inflammatory response in human endotoxemia models may have failed in trials of sepsis, but now are mainstays in the treatment of rheumatoid arthritis, inflammatory bowel disease, ankylosing spondylitis, and gout.108–111 Indeed, endotoxemia protocols have been used safely in humans for decades to test the efficacy of lipopolysaccharide antagonists,112,113 IL-1 receptor antagonists,114,115 IL-10 infusions,116,117 and TNFα blockers118,119 in mitigating the system-wide dysfunction that results from excessive innate immune signaling. Evoked endotoxemia can be used to inform mechanism of action of therapeutics, potentially identifying novel applications, or contraindicating utility. Thalidomide was thought to offer therapeutic benefit through modulation of TNFα; however, results in clinical trials on TNFα modulation were conflicting. In an evoked endotoxemia protocol, thalidomide was found to have no significant effect on the TNFα response to lipopolysaccharide but significantly decreased the IL-6 response, suggesting IL-6 rather than TNFα as a potential target.120 Similarly, evoked endotoxemia has been used to understand the specific in vivo effects of different doses of prednisolone, revealing target effects on fibrinolytic pathways and chemokine responses.121,122 Dobutamine, a catecholamine used to treat septic myocardial dysfunction, has no effect on inflammatory responses to evoked endotoxemia in vivo, despite effects in vitro, highlighting the importance of the human model.123 In our own work, we found no effect of fenofibrate on response to evoked endotoxemia,124 contrasting with a modulating effect of high-dose n-3 polyunsaturated fatty acid supplementation in the same trial.125 In addition to modeling pharmacological interventions, experimental endotoxemia has also been applied to study the capacity of nutrients to modify the systemic inflammatory response. Notably, the anti-inflammatory properties of omega-3 polyunsaturated fatty acids have been assessed by administering fish oil either parenterally126 or through dietary supplementation127,128 before the endotoxin challenge, whereas habitual dietary intake of soy-derived foods may also modify the response to endotoxemia.128

Differences in the evoked responses, and the genetic determinants of these differences, may indeed relate to the clinical course of future disease. For example, our group has shown recently that genetic variation associated with the evoked IL-1RA response during experimental endotoxemia is also predictive of patient survival in septic shock.129 Furthermore, as noted above, evoked endotoxemia revealed a novel genomic locus for the febrile response to lipopolysaccharide (but not resting body temperature), and this locus also associates with outcomes following severe trauma and sepsis.94
common genetic variants influencing both response to evoked endotoxemia and disease risk have been described. A single nucleotide polymorphism in matrix metalloproteinase-8, previously shown to associate with mortality in pneumonia, was found to modulate the inflammatory response to evoked endotoxemia.130 Similarly, genetic variation in fibrinogen and c-reactive protein relate to the endotoxemia response.131,132 Finally, experimental endotoxemia revealed polymorphism-specific effects in TNFα, with the Asp299Gly and Thr399Ile, but not the -308 G/A polymorphisms associating with inflammatory response,133,134 whereas variation in IL6 was not associated with alterations in the IL-6 response to endotoxemia.105 These studies thus highlight common genetic underpinnings of evoked endotoxemia and inflammatory responses, which then direct functional studies and clinical translation.

Limitations and Challenges of the Model

The human experimental endotoxemia model generates a low-grade acute systemic inflammatory state and admittedly does not fully capture chronic subclinical inflammation as is present in cardiometabolic disease. In addition, because of Food and Drug Administration restrictions on the use of experimental endotoxemia in humans, most protocols are now restricted to relatively young (aged, <45 years), healthy, nonobese (body mass index, <30), and nonsmoking individuals. Because of the small number of subjects who have undergone endotoxemia studies and their relatively young age, it has not been possible in this field to date to perform CVD outcome studies and evaluate the relationship between lipopolysaccharide phenotype and future cardiovascular events. In addition, there is also no currently established association between the lipopolysaccharide response and established inflammatory biomarkers of CVD. However, as noted, relative to resting inflammatory biomarker levels (which in fact are modest predictors of CVD), the evoked inflammatory biomarker response to endotoxemia may better reflect the pathophysiology, the genetic underpinnings, and the therapeutic modulation of innate immunity during inflammatory stress. A pragmatic approach to overcome limitations on predictive capacity of the model is to use the endotoxemia model as a tool to focus on specific responses or characteristics of interest and then to assess the relationship of those characteristics to incident clinical disease in independent epidemiological studies.129

Moreover, the single-exposure human endotoxemia model, as approved currently for the use within the United States by the Food and Drug Administration, is unable to capture sustained activation of innate immunity that may occur with chronic or repeated exposures to innate immune ligands (eg, chronic infections, inflammatory bowel disease, or chronic obstructive pulmonary disease). However, repeated-exposure models have been applied by researchers in Europe, further illuminating the biology of chronic innate immune stimulation in clinical disease. Five consecutive days of 2 ng/kg endotoxin administration leads to endotoxin tolerance,135 with evidence of attenuated release of both proinflammatory and anti-inflammatory cytokines over time, leading to less leukocyte and endothelial activation, an effect that may last several weeks.102 In the same model, endothelial dysfunction gradually declines as endotoxin tolerance emerges,136 whereas lipopolysaccharide tolerance does not seem to protect against ischemia–reperfusion injury.137 The repeated-exposure model has also been used for the study of sepsis-induced immunoparalysis, where interferon-γ treatment has partially reversed immune suppression and furthered pharmacological interest for immunostimulation in sepsis.138 Although these findings provide key initial insights into the elements of endotoxin tolerance, much research is still needed to elucidate the role of repeated lipopolysaccharide exposure on cardiometabolic physiology.

Indeed, many compounds, including cytokine pathway modulators, endotoxin antagonists, nutritional supplements, hormones, and novel therapeutics, among others, have, all to varying degrees, been shown to influence the systemic inflammatory response in human experimental endotoxemia.139 However, because of the complexity of the inflammatory response, no single intervention has been shown to blunt the entire inflammatory spectrum during endotoxemia. In fact, TNFα blockers118,119 and IL-1 antagonists14,139 only partially mitigated the systemic inflammatory response in human endotoxemia and were brought to clinical trials of sepsis, in part, because of promising results indicating decreased mortality with such compounds in rodent models of endotoxemia. Recent data suggesting that rodent models of endotoxemia correlate poorly with human conditions may partly explain why these compounds failed in clinical trials of human sepsis.98 Our recent work, however, shows that genetic variation associated with the evoked IL-1RA response to experimental endotoxemia is predictive of patient survival in clinical cohorts with septic shock.129 These data have reignited a discussion on whether IL-1 pathway modulation might provide clinical benefit in sepsis if targeted to subsets of patients with specific genetic or biomarker features (ie, a precision medicine approach). As noted also, TNF pathway blockers and IL-1 antagonists ultimately succeeded in clinical translation and are now mainstays in the treatment of several rheumatological and inflammatory disorders.108-111

Future and Conclusions

Human experimental endotoxemia has established use as a controlled model of systemic inflammation. Coupled with contemporary genomics, transcriptomics, and strategies for development of novel therapeutics, the model provides a unique platform for clinical, genetic, and pharmacological research applications in inflammatory and cardiometabolic diseases (Figure [B]). Controlled sampling within the structure of the experimental model permits cell- and tissue-specific interrogation of genomic, epigenetic, and transcriptomic responses to the activation of innate immunity and may help identify unique molecules or pathways for future treatments that target cell-specific components of innate immunity. Admittedly, human experimental endotoxemia is just one of several complementary approaches, which all possess the advantage of studying genomic and transcriptomic regulation in context.100,101 In all human experimental models, increasing sophistication in multiple omics profiling and
integrative genomics combined with the evoked phenotypic responses allows for the enhanced discovery and profiling of novel pathways and therapeutics even with limited trial sample sizes.\(^{9,12}\) Alongside discovery, the human endotoxemia model allows for the assessment of specific genetic, pharmacological, and lifestyle exposures on cell and organ level responses, as well as the effect of these exposures on the dynamic integrated human host physiology. Finally, in light of recent evidence demonstrating the challenges in extrapolating rodent endotoxemia models to human inflammatory disease,\(^{98}\) greater emphasis on human translational experimental models is warranted.

With the advent of whole exome and genome sequencing, we now have the unique opportunity with the human endotoxemia model to examine the effect of specific loss of function alleles in the human genome on innate immune physiologies and the cell-specific mechanisms underlying the host response in these knockout in vivo. Certainly, an exciting future exists in the controlled, clinically relevant, human endotoxemia model, where precision and personalized initiatives may be discovered, evaluated, and expanded into diagnostic, prognostic, and therapeutic applications.

**Sources of Funding**

This work and related research was supported by National Institutes of Health-National Heart, Lung, and Blood Institute SCCOR Project grant (P50-HL-083799), R01-HL113147, and K24-HL107643 to M.P. Reilly. Dr Ferguson is supported by a postdoctoral fellowship grant from the American Heart Association (12POST11840017). M.P. Reilly is also supported by H111694, DK-090505, and HL-108636.

**References**


**Disclosures**

None.

**Sources of Funding**

This work and related research was supported by National Institutes of Health-National Heart, Lung, and Blood Institute SCCOR Project grant (P50-HL-083799), R01-HL113147, and K24-HL107643 to M.P. Reilly. Dr Ferguson is supported by a postdoctoral fellowship grant from the American Heart Association (12POST11840017). M.P. Reilly is also supported by H111694, DK-090505, and HL-108636.

**References**


Patel et al. Endotoxemia in Modeling Cardiometabolic Disease 533


79. McMahon M, Grossman J, FitzGerald J, Dahlén-E Lee, Wallace DJ, Thong BY, Badsha H, Kahanian K, Charles C, Navab M, Fogelman AM, Hahn BH. Proinflammatory high-density lipoprotein as a biomarker for athero-

80. van Lenten BJ, Hama SY, de Beer FC, Staffordini DM, Mctyre TM, Prescott SM, La Du BN, Fogelman AM, Navab M. Anti-inflammatory HDL becomes proinflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocolu-


85. Pandian AD, Manson JE, Rossouw JE, Siscovick DS, Mouton CP, Rifai N, Wallace RB, Jackson RD, Pettinger MB, Räder PM. Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women’s Health Initiative observa-


87. van Ommen B, van der Grieb J, Ordovas JM, Daniel H. Phenotypic flex-


Inflammation is a key feature of several complex cardiometabolic diseases, and evidence suggests that the activation of the innate immune system may be a contributing factor in the pathophysiology of obesity, insulin resistance, metabolic dyslipidemia, and atherosclerosis. Here, we describe the human experimental endotoxemia model for the specific study of innate immunity in understanding further the pathogenesis of cardiometabolic diseases in humans. In a controlled, experimental setting, administration of an intravenous bolus of purified *Escherichia coli* endotoxin activates innate immunity in healthy human volunteers and elicits specific metabolic consequences that closely resemble those observed chronically in inflammatory cardiovascular disease risk states. Such a model allows for the controlled study of innate immune responses following intravenous endotoxin administration. J Immunol. 1995;155:3038–3045.


**Significance**

Inflammation is a key feature of several complex cardiometabolic diseases, and evidence suggests that the activation of the innate immune system may be a contributing factor in the pathophysiology of obesity, insulin resistance, metabolic dyslipidemia, and atherosclerosis. Here, we describe the human experimental endotoxemia model for the specific study of innate immunity in understanding further the pathogenesis of cardiometabolic diseases in humans. In a controlled, experimental setting, administration of an intravenous bolus of purified *Escherichia coli* endotoxin activates innate immunity in healthy human volunteers and elicits specific metabolic consequences that closely resemble those observed chronically in inflammatory cardiovascular disease risk states. Such a model allows for the controlled study of innate immune influences on cardiometabolic physiology, but perhaps more importantly is uniquely positioned with contemporary technology as a starting point for genomic screening and testing of novel therapeutics for treatment of the inflammatory underpinning of cardiometabolic disease.