Published in final edited form as:

J Theor Biol. 2014 October 7; 358: 132–148. doi:10.1016/j.jtbi.2014.05.036.

Global Sensitivity Analysis of a Mathematical Model of Acute Inflammation Identifies Nonlinear Dependence of Cumulative Tissue Damage on Host Interleukin-6 Responses

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Abstract

The precise inflammatory role of the cytokine interleukin (IL)-6 and its utility as a biomarker or therapeutic target have been the source of much debate, presumably due to the complex pro- and anti-inflammatory effects of this cytokine. We previously developed a nonlinear ordinary differential equation (ODE) model to explain the dynamics of endotoxin (lipopolysaccharide; LPS)-induced acute inflammation and associated whole-animal damage/dysfunction (a proxy for the health of the organism), along with the inflammatory mediators tumor necrosis factor (TNF)- α , IL-6, IL-10, and nitric oxide (NO). The model was partially calibrated using data from endotoxemic C57Bl/6 mice. Herein, we investigated the sensitivity of the area under the damage curve (AUC_D) to the 51 rate parameters of the ODE model for different levels of simulated LPS challenges using a global sensitivity approach called Random Sampling High Dimensional Model Representation (RS-HDMR). We explored sufficient parametric Monte Carlo samples to generate the variance-based Sobol' global sensitivity indices, and found that inflammatory damage was highly sensitive to the parameters affecting the activity of IL-6 during the different stages of acute inflammation. The AUC_{IL6} showed a bimodal distribution, with the lower peak representing

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Conflict of Interest: YV is a co-founder of, and stakeholder in, Immunetrics, Inc. JB is an employee of Immunetrics, Inc. SM and IB declare that they have no conflict of interest.

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healthy response and the higher peak representing sustained inflammation. Damage was minimal at low AUC_{IL6} , giving rise to a healthy response. In contrast, intermediate levels of AUC_{IL6} resulted in high damage, and this was due to the insufficiency of damage recovery driven by anti-inflammatory responses and the activation of positive feedback sustained by IL-6. At high AUC_{IL6} , damage recovery was interestingly restored in some population of simulated animals due to the NO-mediated anti-inflammatory responses. These observations suggest that the host's health status during acute inflammation depends in a nonlinear fashion on the magnitude of the inflammatory stimulus, on the host's propensity to produce IL-6, and on NO-mediated downstream responses.

Keywords

Inflammation Dynamics; Meta-Modeling; Endotoxin; Cytokines; Nitric oxide

1. Introduction

1.1 Modeling acute inflammation

Acute inflammation is the initial response of the body to various biological stresses, including bacterial infection and tissue injury. Inflammation is a multi-scale process manifesting at the molecular, cellular, tissue, organ, and whole-organism levels. The action of immune cells and secreted molecules combat the offending insult and also trigger a repair process to allow the body to return to pre-insult homeostasis. Properly regulated inflammation allows for timely recognition and effective reaction to injury or infection, but diseases such as sepsis involve disordered inflammation that in turn impairs physiological functions (An et al, 2012; Vodovotz and Billiar, 2013).

The complex, non-linear nature of inflammation has made it difficult to directly translate results from animal studies to clinical trials (Neugebauer et al, 2001; Vodovotz and Billiar, 2013; Vodovotz et al, 2008). Several computational representations of the acute inflammatory response developed in the recent years have provided tools for gaining insights into and making testable predictions of this complex biological process (Parker and Clermont, 2010; Vodovotz and An, 2009; Vodovotz and Billiar, 2013; Vodovotz et al., 2004; Vodovotz et al, 2008; Vodovotz et al., 2009). Various frameworks have been used successfully to represent inflammation computationally; for example, equation-based models such as difference and differential equations (Scheff et al, 2013; Vodovotz and Billiar, 2013; Vodovotz et al., 2004; Vodovotz et al, 2009), data-driven models (An et al, 2012; Croft et al, 2012; Vodovotz and Billiar, 2013; Vodovotz et al, 2009), and agent-based models (An et al, 2012; Vodovotz and An, 2009; Vodovotz and Billiar, 2013). Differential equation-based representations can adequately address the complex, non-linear, dynamic interactions characterizing a well-mixed system, especially when such models are based on data obtained in bio-fluids such as serum or plasma (An et al, 2012; Scheff et al, 2012).

1.2 Analysis of large-scale ODE models for inflammation

1.2.1 Current models of acute inflammation—In our earlier models of acute inflammation, we studied the role of a small number of key mediators using techniques such

as bifurcation analysis (Kumar et al, 2004; Reynolds et al, 2006). With further advances in experimental models of inflammation, species-specific parameters were determined to explain differences in the dynamics of inflammation with changes in the sources of inflammation as well as the primary drivers of inflammation (Daun et al, 2008b; Nieman et al, 2012; Vodovotz et al., 2006). This led to large-scale ODE models containing a large number of distinct mediators as well as local and systemic compartments (Chow et al, 2005; Nieman et al, 2012; Torres et al., 2009). These models have been successful in explaining the dynamics of various mediators as well as the identification of processes responsible for various clinical outcomes of inflammation (Nieman et al, 2012; Vodovotz and Billiar, 2013; Vodovotz et al, 2008).

1.2.2 Analyzing ODE behavior using sensitivity analysis—Inflammation can either resolve or become self-sustaining, depending on a complex interaction between the original insult and host factors (Medzhitov, 2008; Nathan, 2002). Analysis and extraction of key mechanisms that drive inflammation into different clinically relevant regimes, however, has been difficult for high-dimensional ODE models. The challenges arise from two sources, namely the uncertainty of the model parameters and the computational burden of exploring the influence of a large number of parameters and their correlations. To overcome these challenges, large-scale ODE models have to employ supplementary tools like sensitivity and uncertainty analysis, model identifiability analysis and parameter set reduction in the modeling loop (Chu and Hahn, 2012; Jayaraman and Hahn, 2009; Kiparissides et al, 2011; Vodovotz et al, 2009).

Sensitivity analysis (SA) is an increasingly important step in this process and is often the first step in learning the nature of different parameters in the model (Marino et al, 2008; Sahle et al, 2008; Saltelli et al, 2008). SA can also guide other processes like parameter estimation in the modeling loop by providing importance measures to the model parameters (Kiparissides et al, 2009). In general, SA can be carried out by two approaches: local and global. Local methods study the influence of single parameter in isolation and the other parameters are kept constant at their nominal values. Global methods study the influence of a parameter by varying it in a defined direction and also simultaneously varying the other parameters in a random fashion in the entire parameter space (Saltelli et al, 2008). While local methods are valid near the nominal region of the parameter space, global methods are independent of the nominal values and also provide a complete mapping of the input-output (IO) behavior of the model (Sobol', 2001). Given the nature of large-scale inflammation models, it is necessary to apply global sensitivity analysis (GSA) tools to identify and validate key mechanisms predicted by the models. These methods also hold the potential to help in the classification of mechanisms that can lead to clinically distinct outcomes such as resolution vs. self-maintenance of inflammation.

The traditional methods of GSA are variance decomposition schemes and they employ computationally expensive exploration of the parameter space using different sample generation techniques (Sobol', 2001). Sobol' and FAST algorithms are two popular techniques for variance-based GSA (Saltelli et al, 1999). The advantages of these algorithms are, however, challenged by the large number of Monte Carlo (MC) samples ($\sim 10^5$) necessary for complete identification of the IO space of the model (Sobol', 1998). This

aspect renders the use of a detailed parametric analysis of the large-scale ODE expensive, and thereby restricts the modeler to fairly simple local analysis.

The computational cost for evaluating GSA can be reduced in two ways: reducing the time required for evaluating the model at a single point in the parameter space, and reducing the number of samples required for GSA. A common method is model order reduction, which employs various approximations to “reduce” the original complex ODE model to a reduced set of ODEs containing only key model species (Hahn and Edgar, 2002; Okino and Mavrovouniotis, 1998; Schilders et al, 2008). These approximations may employ lumping of the species, removal of non-sensitive species, or quasi steady state assumptions of the fast time-scale reactions (Okino and Mavrovouniotis, 1998). An alternate strategy is the meta-modeling approach, which “replaces” the entire complex ODE model with a set of approximation functions that capture accurate model dynamics over a prescribed parameter space (Barton, 1992; Barton, 1998). The essential difference between the two methods is that while reduced-order models retain the original mechanistic information, they are designed to lose non-essential information. Meta-models, on the other hand, do not represent mechanism. They are designed to represent the model dynamics accurately over a specified sampled space.

1.2.3 A Meta-model approach for global sensitivity analysis—In the present study, we sought to address the difficulty of GSA for large-scale ODE models, such as our models of the acute inflammatory response, using a global meta-modeling technique known as Random Sampling High Dimensional Model Representation (RS-HDMR). RS-HDMR is an efficient method for estimating variance based Sobol' sensitivity indices accurately, at a lower computational cost (Li and Rabitz, 2012) and the validity of the method has been proven for many physical and engineering models. Furthermore, the function approximations constructed using RS-HDMR give a direct way of studying the direction of influence of each studied sensitive parameter.

1.3 Goals of the current study

In the current study, our primary goal was to evaluate the outcomes of LPS challenge in *in silico* representations of experimental models in mice. We started from a zero LPS load state in which the system shows minimal concentration of cytokines and immune cells. We aimed to evaluate the effect of variations in the dynamics of the inflammatory processes on the final outcome. With the goal of defining key determinants of inflammation that might lead to sustained vs. resolving inflammatory damage, we used RS-HDMR to analyze the global IO behavior of a nonlinear ODE model of acute inflammation developed in (Chow et al, 2005; Torres et al, 2009). This model has 15 state variables and 51 rate constants. The model describes the interactions of key mediators in acute phase inflammation in mice subjected to various doses of endotoxin. Using RS-HDMR, we evaluated the sensitive parameters affecting cumulative tissue damage that stands as a global outcome of systemic inflammation. Through our analysis, we identified this outcome to be primarily dependent on the parameters affecting IL-6 levels, followed by parameters governing the production of reactive nitric oxide (NO) levels. Consolidating the results from our RS-HDMR analysis, we identified the nonlinear dependence of the state of inflammation on the interactions among

three major drivers of outcome in this model: inflammatory damage, IL-6 and NO. We suggest that this methodology could be applied more broadly to similarly nonlinear models in biology.

2. Methods

2.1 Experimental procedures

The experimental data used in the model building cycle are described in detail elsewhere (Chow et al, 2005; Torres et al, 2009). The salient features of these experiments are mentioned below.

2.1.1 Animal Model—The experiments were conducted in C57Bl/6 mice (6-10 weeks old; Charles River Laboratories, Charles River, ME). The experiments were conducted according to National Institutes of Health Guidelines on the Use of Laboratory Animals. For the experimental paradigm used for this study, the mice were subjected to LPS (from *E. coli* O111: B4, 3, 6, or 12 mg/kg intraperitoneally; Sigma Chemical Co., St. Louis, O) or saline control.

2.1.2 Analysis of cytokines and NO₂⁻/NO₃⁻—The cytokines TNF-α, IL-6 and IL-10 were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN). Nitric oxide was measured as NO₂⁻/NO₃⁻ by the nitrate reductase method using a commercially available kit (Cayman Chemical, Ann Arbor, MI).

2.2 Mathematical model of acute inflammation

The model described herein, which was published previously (Torres et al, 2009), consists of a system of 15 ODEs, in which inflammation is initiated by pathogen-derived products (such as LPS), trauma (i.e. surgical preparation/sham operation) and/or hemorrhagic shock due to massive blood loss. The model is structured as follows:

$$M'_R = - \left[\left(k_{MLPS} \frac{LPS(t)^2}{1 + (LPS(t)/x_{MLPS})^2} + k_{MD} \frac{D^4}{x_{MD}^4 + D^4} \right) \times \left(\frac{TNF^2}{TNF^2 + x_{MTNF}^2} + k_{M6} \frac{IL6^2}{x_{M6}^2 + IL6^2} \right) + k_{MTR} TR(t) + k_{MB} f_B(B) \right] \frac{1}{1 + (IL10 + \dots)}$$

$$M'_A = \left[\left(k_{MLPS} \frac{LPS(t)^2}{1 + (LPS(t)/x_{MLPS})^2} + k_{MD} \frac{D^4}{x_{MD}^4 + D^4} \right) \times \left(\frac{TNF^2}{TNF^2 + x_{MTNF}^2} + k_{M6} \frac{IL6^2}{x_{M6}^2 + IL6^2} \right) + k_{MTR} TR(t) + k_{MB} f_B(B) \right] \frac{1}{1 + ((IL10 + \dots))}$$

$$N'_R = - \left[\left(k_{NLPS} \frac{LPS(t)}{1 + (LPS(t)/x_{NLPS})} + k_{NTNF} \frac{TNF^2 x_{TNF}^2}{TNF^2 + x_{TNF}^2} \right) + \left(k_{N6} \frac{IL6^2}{1 + (IL6/x_{N6})^2} + k_{ND} \frac{D^2}{1 + (D/x_{ND})^2} \right) + k_{NTR} TR(t) + k_{NB} f_B(B) \right] \frac{1}{1 + (IL10 + \dots)}$$

$$N'_A = \left[\left(k_{NLPS} \frac{LPS(t)}{1+(LPS(t)/x_{NLPS})} + k_{NTNF} \frac{TNF^2 x_{TNF}^2}{TNF^2 + x_{TNF}^2} \right) + \left(k_{N6} \frac{IL6^2}{1+(IL6/x_{N6})^2} + k_{ND} \frac{D^2}{1+(D/x_{ND})^2} + k_{NTR} TR(t) + k_{NB} f_B(B) \right) \right] \frac{1}{1+}$$

$$iNOSd' = \left[k_{iNOSN} N_A + k_{iNOSM} M_A + k_{iNOSEC} \left(\frac{TNF^2}{1+(TNF/x_{iNOSTNF})^2} + k_{iNOS6} \frac{IL6^2}{1+(IL6/x_{iNOS6})^2} \right) \right] \frac{1}{1+(IL10/x_{iNOS10})^4} \\ \times \frac{1}{1+(NO/x_{iNOSNO})^4} - k_{iNOSd} iNOSd$$

$$iNOS' = k_{iNOS} (iNOSd - iNOS)$$

$$NO_3' = k_{NO3} (NO - NO_3)$$

$$eNOS' = k_{ENOSEC} \frac{1}{1+(TR(t)/x_{ENOSTR})^4} \frac{1}{1+LPS(t)/x_{ENOSLPS}} \times \frac{1}{1+TNF/x_{ENOSTNF}} - k_{eNOS} eNOS$$

$$TNF' = (k_{TNFN} N_A + k_{TNFM} M_A) \frac{1}{1+((IL10+CA)/x_{TNF10})^2} \frac{IL6}{1+(IL6/x_{TNF6})^3} - k_{TNF} TNF$$

$$IL12' = k_{i2M} M_A \frac{1}{1+(IL10/x_{i210})^2} - k_{i2} IL12$$

$$IL6' = (k_{6N} N_A + M_A) \left[k_{6M} + k_{6TNF} \frac{TNF^2}{TNF^2 + x_{6TNF}^2} + k_{6NO} \frac{NO^2}{x_{6NO}^2 + NO^2} \right] \frac{1}{1+((IL10+C_A)/x_{610})^2} + k_6 (S_6 - IL6)$$

$$IL10' = (k_{10N} N_A + M_A (1 + k_{10A} A(t))) \left[k_{10MR} + k_{10TNF} \frac{TNF^4}{TNF^4 + x_{10TNF}^4} + k_{106} \frac{IL6^4}{x_{106}^4 + IL6^4} \right] \left[(1 - k_{10R}) \frac{1}{1+(IL12/x_{1012})^4} + k_{10R} \right] \\ - k_{10} (IL10 - S_{10})$$

$$CA' = k_{CATR} A(t) - k_{CA} CA$$

$$D' = \left[k_{DE} f_B(B) + k_{D6} \frac{IL6^4}{IL6^4 + x_{D6}^4} \right] \frac{x_{DNO}^2}{x_{DNO}^2 + NO^2} - k_D D$$

$$B' = Active(t, t_{bon}, t_{drop}) Hem(t) + (1 - Active(t, t_{bon}, t_{drop})) k_B$$

$$\left[\frac{1}{1 + k_{BNO}(iNOs + eNOS - x_{BNO})} - B \right]$$

where,

$$NO = iNOs(1 + k_{NOMA}(M_A + N_A)) + eNOS$$

$$LPS(t) = LPS(0) \exp(-k_{LPS} t)$$

$$TR(t) = TR_{ON} \exp\left(-(t - t_{TR})^2 / x_{TR}^2\right)$$

$$A(t) = A_{ON} \exp\left(-(t - t_A)^2 / x_A^2\right)$$

$$f_B(B) = H(B_0 - B)(B_0 - B)^4$$

$$Hem(t) = -kbh / (t_{drop} - t_{bon})$$

$$Active(t, start, stop) = H(t - start) - H(t - stop)$$

$$H(x) = \begin{cases} 1, & x > 0 \\ 0, & x < 0 \end{cases}$$

The model components are described in Table 1 in the text. Model parameters were published previously (Torres et al, 2009), and are presented here in Tables A1-A3. Additional details of the development of this mathematical model are published elsewhere (Chow et al, 2005; Lagoa et al, 2006; Prince et al, 2006). The *FORTTRAN* code for the ODE model is provided online.

The original model was calibrated to experimental data from three different inflammatory paradigms (Chow et al, 2005): (a) bacterial lipopolysaccharide (LPS), which induces a sepsislike acute inflammatory response, at doses of 3, 6 and 12 mg/kg ip; (b) 2.5h of sham (surgical cannulation) trauma alone; and (c) cannulation followed by 2.5 h of HS at a blood pressure of 25-30 mmHg (approximately a 70% drop from baseline mean arterial pressure, in some cases followed by various times of resuscitation). We have suggested that a single mathematical model, with diverse inputs corresponding to LPS, surgical cannulation, and HS could reproduce the dynamics of acute inflammation in young mice (Chow et al, 2005) and modifications of this model's parameters could account for inflammatory responses in aged mice (Namas et al, 2013). The calibrated parameter values for young mice are referred to as the “nominal” values of the parameters in this manuscript. The initial conditions determine different experimental paradigms. For the case of endotoxemia analyzed in this study,

$TR_{ON} = 0$, $A_{ON} = 0$, $B(0) = 1$, $t_{bon} = 0$, $t_{drop} = 0$. For this reason, the equation for blood pressure reduces to the form:

$$B' = k_B \left[\frac{1}{1 + k_{BNO}(iNOS + eNOS - x_{BNO})} - B \right]$$

2.3 Global Sensitivity Analysis using RS-HDMR

2.3.1 Sample generation—GSA evaluates the sensitivity of model parameters on the model output. For detailed ODE models, the common model parameters include the kinetic rate parameters and the initial conditions. Since our aim was to evaluate the influence of changes in the kinetic rate parameters, they were chosen as the free model “input” parameters (summarized in Table A1) and the other parameters, including most of the initial conditions (except LPS concentration), saturation constants, and source terms (summarized in Tables A2-A3) were kept constant. Therefore, the 51 kinetic rate parameters and the initial LPS concentration, together form 52 input free parameters of the model for the current analysis. A set of MC samples was generated from these input parameters for 100-fold variations around the nominal values of the rate parameters. Simulated LPS concentration was varied in the range 0 - 20 mg/kg. The list of all the free parameters (Ξ_i) and their actual ranges ($[a_i, b_i]$) is given in Table A1. For RS-HDMR, the input parameters must be normalized (say into x_i) so that they lie in the range [0,1]. The normalized variable, x_i , can be converted into its actual value, Ξ_i , in the interval $[a_i, b_i]$ by the transformation: $\Xi_i = a_i + (b_i - a_i) \times x_i$ which is then used to evaluate the ODEs. The model was simulated in FORTRAN R90. Random samples were generated using the *ran* function in *FORTRAN*, which generates a uniform random number between 0 and 1 (Teukolski et al, 1989) with a new seed for each MC sample set. Each such sample is denoted by vector $\bar{x}^s = (x_1^s, x_2^s, \dots, x_k^s)$, where individual components $x_i \in [0,1]$, k denotes the number of free parameters, and the superscript, s , denotes the sample number. The resulting MC samples

$$M_{train} = \begin{bmatrix} \bar{x}^1 \\ \bar{x}^2 \\ - \\ - \\ \bar{x}^N \end{bmatrix} = \begin{bmatrix} x_1^1, x_2^1, \dots, x_k^1 \\ x_1^2, x_2^2, \dots, x_k^2 \\ - \\ - \\ x_1^N, x_2^N, \dots, x_k^N \end{bmatrix},$$

are collected in the training matrix, where N is the total number of samples. For the generated matrix, M_{train} , the original ODE model described in the previous section was integrated using the DLSODE solver (Hindmarsh, 1983). The complete integration process for 10^5 samples takes 4 min on INTEL® Core™ 2 Quad CPU (Q8400 @ 2.66GHz).

2.3.2 RS-HDMR algorithm for meta-model development—RS-HDMR is an efficient technique for the identification of the nonlinear IO behavior of high dimensional systems (Li and Rabitz, 2012; Li et al, 2001). The performance characteristics of the method was verified in high-dimensional ODE models from different fields, namely atmospheric photochemistry (Li et al, 2002b), genetic circuits (Feng et al, 2004), combustion processes (Davis et al, 2011), signaling pathways (Mathew et al, 2014) and in network identification of a biochemical interaction model (Miller et al., 2012). RS-HDMR decomposes the selected output of the ODE, $Y = f(x)$ (here, AUC_D and AUC_{IL6}), into component functions represented by the following hierarchical expansion in the k input parameters $x = (x_1, x_2, \dots, x_k)$:

$$Y = f(\bar{x}) = f_0 + \sum_{1 \leq i \leq k} f_i(x_i) + \sum_{1 \leq i \leq j \leq k} f_{ij}(x_i, x_j) + \dots + f_{123\dots k}(x_1, x_2, \dots, x_k) \quad (1)$$

The individual functions are called component functions and represent the influence of each type of input combination. These functions are evaluated from specific instances of the ODE output as detailed below:

$$\begin{aligned} f_0 &= E(Y) \\ f_i(x_i) &= E(Y|x_i) - f_0 \\ f_{ij}(x_i, x_j) &= E(Y|x_i, x_j) - f_i - f_j - f_0 \text{ etc.} \end{aligned} \quad (2)$$

The component functions are, therefore, higher dimensional integrals defined by:

$$\begin{aligned} E(Y) &= \int_{[0,1]^k} f(\bar{x}) d\bar{x} \\ E(Y|x_i) &= \int_{[0,1]^{k-1}} f(\bar{x}) \prod_{m \neq i} dx_m \text{ etc.} \end{aligned} \quad (3)$$

Each component function represents the contribution of the corresponding input variable to the output. First order components, $f_i(x_i)$, describe the contribution of a single parameter independently of the others while higher order functions, $f_{ij}(x_i, x_j)$ etc., describe the joint contributions of the parameters. Most importantly, the method rests on the realization that the expansion given in Equation (1) often converges rapidly so that higher order interactions (3) are often negligible. Expansions until the second-order terms are known to sufficiently describe most physical systems (Li et al, 2001; Li et al, 2002b).

The component functions can be evaluated by polynomial approximations (Li and Rabitz, 2012; Li et al, 2002a; Li et al, 2010). In this work, we used orthonormal polynomial approximations for 2nd order RS-HDMR given by:

$$\begin{aligned} f_i(x_i) &\approx \sum_{1 \leq p \leq u} \alpha_p^i \phi_p^i(x_i) \\ f_{ij}(x_i, x_j) &\approx \sum_{1 \leq q \leq v} \sum_{1 \leq r \leq w} \beta_{qr}^{ij} \phi_q^i(x_i) \phi_r^j(x_j) \end{aligned} \quad (4)$$

The integers u, v, w are orders of the orthonormal polynomials, ϕ , and are usually 3 (Li et al, 2010). Based on Li *et al.*, we used Jacobi polynomials in the domain [0,1] as the orthonormal basis functions, ϕ . These polynomials satisfy the following properties of orthonormality, namely,

$$\begin{aligned} \int_0^1 \phi_a(x) dx &= 0, \quad a = 1, 2, 3 \\ \int_0^1 \phi_b^2(x) dx &= 1, \quad b = 1, 2, 3 \\ \int_0^1 \phi_c(x) \phi_d(x) dx &= 0, \quad c \neq d \end{aligned}$$

Using these properties, the first three orthonormal polynomials were constructed to be:

$$\begin{aligned} \phi_1(x) &= \sqrt{3}(2x - 1) \\ \phi_2(x) &= 6\sqrt{5}\left(x^2 - x + \frac{1}{6}\right) \\ \phi_3(x) &= 20\sqrt{7}\left(x^3 - \frac{3}{2}x^2 + \frac{3}{5}x - \frac{1}{20}\right) \end{aligned} \quad (5)$$

See Rice *et al.* for further description of the construction of Jacobi polynomials (Rice and Do, 2012). By definition, the orthonormality of the basis functions preserve the orthonormality between the RS-HDMR component functions. Hence, the coefficients in Equation (4), α_p^i and β_{qr}^{ij} are obtained by:

$$\begin{aligned} \alpha_p^i &= \frac{\int_{[0,1]^k} f(\bar{x}) \phi_p(x_i) d\bar{x}}{\int_{[0,1]} \phi_p^2(x_i) dx_i} \approx \frac{1}{N} \sum_{s=1}^N f(\bar{x}^s) \phi_p(x_i^s), \\ \beta_{qr}^{ij} &= \frac{\int_{[0,1]^2} f(\bar{x}) \phi_q(x_i) \phi_r(x_j) d\bar{x}}{\int_{[0,1]^2} \phi_q^2(x_i) \phi_r^2(x_j) dx_i dx_j} \approx \frac{1}{N} \sum_{s=1}^N f(\bar{x}^s) \phi_q(x_i^s) \phi_r(x_j^s) \end{aligned} \quad (6)$$

Using MC integration approximation, these coefficients can be estimated by least squares regression on the model output, Y , obtained for the training matrix, M_{train} (Li et al., 2001). The mean output is obtained by approximating the integrals using summation terms,

$$f_0 \approx \frac{1}{N} \sum_{s=1}^N f(\bar{x}^s). \text{ It is important to note that the entire coefficient in Equation (6) can be}$$

obtained simultaneously from a single set of MC samples. This leads to a substantial reduction in the computational cost due to the elimination of repeated sampling. As seen from most physical models, the number of samples required for accurate estimation of the coefficients is of the order of 10^3 (Li and Rabitz, 2012).

Using Equations (4)-(6), the terms $\sum_i f_i(x_i) + \sum_{i,j} f_{ij}(x_i, x_j)$ amount to $\tilde{f}(\bar{x})$, which is an estimate predicted by RS-HDMR of $f(\bar{x})$ from Equation (1). It is essential to compare how well $\tilde{f}(\bar{x})$ represents $f(\bar{x})$. For this, we generated MC samples and evaluated the model output $Y = f(\bar{x})$ directly from the ODE and from the second order RS-HDMR based prediction, $\tilde{f}(\bar{x})$ and estimated the coefficient of determination (R^2) using the relation:

$$R^2 = 1 - \frac{\sum_{s=1}^N [f_s(\bar{x}) - \tilde{f}_s(\bar{x})]^2}{\sum_{s=1}^N [f_s(\bar{x}) - f_0]^2} \quad (7)$$

The numerator of the second term in Equation (7) denotes the sum of squares of residuals and the denominator denotes the total sum of squares (hence, proportional to the total variance).

2.3.3 Sobol' sensitivity indices—We used the variance decomposition method to estimate the global sensitivity indices (or Sobol' indices). The Sobol' indices present the sensitivity of the output to specific perturbations of the input parameters. These indices are of various orders based on the number of parameters whose effects are studied. For example, first-order indices show the contribution of individual parameters, the second-order indices show the contributions of pairs of parameters, etc. Each index represents a fraction of the total variance. If σ^2 is the total variance of the model output in $[0,1]^k$, $Y = f(\bar{x})$, the decomposition of total variance assuming that contributions from higher order (3) are negligible is given by:

$$\sigma^2 = \sum_{1 \leq i \leq k} \sigma_i^2 + \sum_{1 \leq i < j \leq k} \sigma_{ij}^2 \quad (8)$$

Here, each term on the right hand side of Equation (8) signifies the “independent” contribution of the particular parameter combination. The total variance of the model output in k -dimensional space is estimated by the relation,

$$\sigma^2 = \int_{[0,1]^k} (f(\bar{x}) - f_0)^2 d\bar{x} \quad (9)$$

By definition, the first order and second order Sobol' indices can be related to the variance by the relations:

$$S_i = \frac{\sigma_i^2}{\sigma^2} \text{ and } S_{ij} = \frac{\sigma_{ij}^2}{\sigma^2} \quad (10)$$

The relations in Equation (10) show that the Sobol' index is a fraction of the total variance that is explained by the variance in the selected parameter combination.

2.3.3.1 Direct MC based evaluation of Sobol' indices: The estimation of Sobol' indices using direct Monte Carlo integration requires repeated sampling from the parameter space and therefore, is computationally expensive (Feil et al., 2009). For evaluation of Sobol' indices as given in Equation (10), the individual variances have to be evaluated first. The individual variances in Equation (8) can be obtained by:

$$\begin{aligned} \sigma_i^2 &\approx \frac{1}{N-1} \sum_{s=1}^N \left(f(x_1^s, x_2^s, \dots, x_k^s) \times f(x_1^{s'}, x_2^{s'}, \dots, x_i^{s'}, \dots, x_k^{s'}) \right) - f_0^2 \text{ and} \\ \sigma_{ij}^2 &\approx \frac{1}{N-1} \sum_{s=1}^N \left(f(x_1^s, x_2^s, \dots, x_k^s) \times f(x_1^{s'}, x_2^{s'}, \dots, x_i^s, \dots, x_j^s, \dots, x_k^{s'}) \right) - f_0^2 \end{aligned} \quad (11)$$

The summation term in Equation (11) contains a product of the ODE based outputs obtained for two different MC samples, s and s' . For the second term of the product, all the variables except the ones under study are resampled from another MC matrix. The total variance is approximated as:

$$\sigma^2 \approx \frac{1}{N-1} \sum_{s=1}^N f^2(\bar{x}^s) - f_0^2 \quad (12)$$

In Equations (10)-(12), the functions, $f(\bar{x}^s) \equiv f(x_1^s, x_2^s, \dots, x_k^s) \equiv Y$ and are hence, obtained directly from the ODE outputs. Note that these functions are different from the individual component functions ($f_i(x_i), f_{ij}(x_i, x_j)$) of RS-HDMR.

2.3.3.2 RS-HDMR based evaluation of Sobol' indices: The RS-HDMR component functions present a convenient way to calculate all the Sobol' indices using a single set of samples like M_{train} (Li et al., 2001; Li et al., 2002b). The orthogonality of the RS-HDMR component functions allows the estimation of individual variances that are contributed independently (first-order) and jointly (higher order) by the input parameters. Using the component function definitions, the variance decomposition can now be written as:

$$\sigma^2 = \sum_{1 \leq i \leq k} \int_{[0,1]} f_i^2(x_i) dx_i + \sum_{1 \leq i < j \leq k} \int_{[0,1]^2} f_{ij}^2(x_i, x_j) dx_i dx_j \quad (13)$$

and consequently, the Sobol' indices can be written as:

$$S_i = \frac{\int_{[0,1]^1} f_i^2(x_i) dx_i}{f_0^2} \text{ and } S_{ij} = \frac{\int_{[0,1]^2} f_{ij}^2(x_i, x_j) dx_i dx_j}{f_0^2} \quad (14)$$

Further, using the coefficients obtained described in Section 2.3.2, simple relationships can be obtained for the Sobol' indices:

$$S_i = \frac{\sum_{1 \leq p \leq u} (\alpha_p^i)^2}{f_0^2} \text{ and } S_{ij} = \frac{\sum_{1 \leq q \leq v} \sum_{1 \leq r \leq w} (\beta_{qr}^{ij})^2}{f_0^2} \quad (15)$$

As discussed in Section 2.3.2, the evaluation of the coefficients in Equation (15) requires only one set of MC samples, and by extension, this means that all the Sobol' indices (first and second order) can be obtained simultaneously. This is unlike the case for direct MC based evaluation in Equations (11)-(12), where a new set of samples must be generated for each index and hence, is dependent on the total number of parameters and their combinations. Our prime goal is to evaluate the Sobol' indices using RS-HDMR. However, direct MC based indices are also evaluated to compare the accuracy of the RS-HDMR estimates with the direct MC estimates (Section 3.3). For direct MC based evaluation of the Sobol' indices, a large number of samples ($\sim 10^5$ - 10^6) are required (Feil et al, 2009). For RS-HDMR based evaluation of the indices, lower number of samples ($\sim 10^3$) is sufficient. However, for comparison between the direct MC based indices and RS-HDMR based indices, we chose 10^5 samples in all the plots of this manuscript. We ensured that all the indices reached convergence by 10^5 samples and remained unchanged by further increase in sampling size.

3. Results

3.1 Mediators of acute inflammation during endotoxin challenge in mice

The nonlinear ODE model used in the present study has been described previously (Chow et al, 2005; Torres et al., 2009). This model was calibrated initially using multiple experimental scenarios of acute inflammation in C57Bl/6 mice, namely endotoxemia, surgical cannulation trauma, and cannulation trauma combined with hemorrhagic shock. The model contains kinetic information about several well-accepted constituents (molecules, cells, and physiologic processes) involved in acute inflammation (see Table 1 and Section 2.2).

In the present study, we focused solely on endotoxemia challenge, i.e. acute inflammation induced by Gram-negative bacterial LPS. Endotoxemia is a canonical, well-studied acute inflammation paradigm that mimics some aspects of Gram-negative sepsis (Beutler and Rietschel, 2003; Parker and Watkins, 2001) (though endotoxemia differs from sepsis in that there is no dividing pathogen that helps drive inflammation). Our aim was to explore the interplay among the key cellular and molecular components of the acute inflammatory response, which in our model is intertwined with physiology (blood pressure) and “health status” of the animal (a variable we term “damage”). At the molecular level, the damage

term in our model shares some characteristics with endogenous Damage-Associated Molecular Pattern (DAMP) molecules (Matzinger, 2002), which are host-derived molecules that are released from stressed or damaged cells and stimulate inflammatory cells to produce classical inflammatory cytokines. The cellular components include the macrophages and neutrophils in two states, namely resting and active. The proximal molecular components include an explicitly pro-inflammatory cytokine, TNF- α (Beutler and Cerami, 1989; Croft et al, 2012); an explicitly anti-inflammatory cytokine, IL-10 (Bogdan et al, 2000; Hofmann et al, 2012); and various other cytokines that modulate the process (e.g. IL-12 (Lyakh et al., 2008; Romani et al, 1997)). A key molecule included in our model is IL-6, which can drive both pro- and anti-inflammatory actions (Mihara et al, 2012). We also include the enzyme inducible NO synthase (iNOS) and endothelial NO synthase (eNOS). These mediators produce stable NO reaction products locally, which can be either protective or detrimental, depending on context (Salvemini et al, 2001).

Different levels of inflammation-induced damage can be correlated to clinically relevant outcomes; in particular, damage which recovers is an indicator of a healthy inflammatory response (Chow et al, 2005). Damage (like DAMPs) also stimulates further inflammation, thus resulting in a long-range positive feedback that results in a self-sustaining inflammation. Such an effect is clinically associated with multiple organ dysfunction syndrome (MODS) (An et al, 2012; Chow et al, 2005; Gallucci and Matzinger, 2001), and this positive feedback loop of inflammation $\rightarrow$ damage $\rightarrow$ inflammation is a central feature of the mathematical models of inflammation (Vodovotz and Billiar, 2013; Vodovotz et al, 2008).

Since damage in essence integrates inflammation and the health status of the individual, we analyzed the systemic response to inflammation with the help of a global parametric analysis, using damage (D) as an index to identify the inflammatory mechanisms that underpin diverse health outcomes. We assessed the cumulative effect of changes in parameter values on D , by examining the area under the damage curve (AUC_D) until 28 h of simulated time. The AUC_D response was recorded while globally varying all the rate parameters (k 's) around their nominal values, with the simulated LPS concentration varied in the range 0-20 mg/kg (see Tables A1-A3).

3.2 Effect of initial LPS dose on overall tissue damage

Our global input perturbations can be considered as experimentally relevant presentation of acute inflammation in mice with different genetic or physiological makeup injected with different LPS doses. The influence of the input perturbations on damage is shown in Figure 1A as a histogram of the AUC_D for 10^5 Monte Carlo samples in the 52-dimensional space (51 rate parameters and 1 initial concentration of LPS). Since the distribution of the parameters is not known *a priori*, each parameter is chosen independently from a uniform distribution as is typically done in GSA (Saltelli et al, 2008). AUC_D distribution reached convergence by 10^5 samples, and hence we chose to select 10^5 samples for further analysis. Out of the 10^5 samples in the distribution, 78% of the sampled values were observed to lie in the lower bins (< 300), indicating that most of the parameter combinations tend to result in low damage. At the same time, very high values of AUC_D (> 1000) were also observed for

3% of the samples, even by 28 h. Under nominal parameters, this level of damage requires high doses of LPS (> 9 mg/kg) and a relatively long stimulation time of 96 h (Chow et al., 2005). Current parametric analysis shows that these damage levels may be reached early. Typically, LPS doses in the range 15-30 mg/kg have been shown to result in high mortality in C57Bl/6 mice (Chow et al., 2005; Wakahara et al., 2005); which translates to AUC_D of 250 under nominal conditions of the current model. Hence, $AUC_D > 250$ may already be lethal in some mice. The correspondence between LPS concentration and AUC_D was further explored in Figure 1B, where the parametric simulations of AUC_D (blue scatter points) were plotted against the administered LPS dose, from 0.001 – 20 mg/kg. We find that below 0.02 mg/kg of LPS, the AUC_D remains very low (10^{-5} to 10^{-3}) for all parameter combinations. The effect of LPS is first felt between 0.02 to 0.1 mg/kg. It is only above 0.1 mg/kg LPS concentration that a shift to high AUC_D is seen with increasing LPS concentration. In particular, above 0.1 mg/kg of LPS administration, $AUC_D > 500$ were observed.

Interestingly, these doses of LPS were well below 12 mg/kg, which was identified as a lethal dose characterized by persistent IL-6 levels and high damage, also shown in Figure 1B by the red curve. For doses greater than 12 mg/kg, Chow *et al.* showed that the damage curve rose rapidly and failed to resolve within 24 hr (Chow et al., 2005). Parallel literature reports a broad range of lethal doses of LPS administration for similar treatment duration, for example, ~ 50 mg/kg (LD_{100}) by (Purswani et al, 2002) and 10-30 mg/kg (LD_{10} and LD_{90} at 24 h) by (Wakahara et al., 2005) for the same strain of mice. Figure 1B further shows that even at very high LPS concentrations, the low damage regions still remain populated, in support of reported differences as arising from the different levels of physiological response mounted by the host (Foteinou et al, 2009b). Hence, the model predictions as well as the literature observations show the importance of host-specific factors. Even though the initial concentration of LPS sets the acute inflammatory response in motion, the final outcome may very well depend on the physiological response of the host. For identification of the major interactions causing such varying responses, a GSA of AUC_D with the selected 52 parameters was performed next.

3.3 Identification of key mediators of tissue damage using global sensitivity analysis

As established from the parametric analysis, the output of the system of ODEs is highly dependent on the rate constants as well as the initial LPS concentrations. For a highly nonlinear model, it is important to study the influence of the global variations in these input variables on the output. Such a global analysis, however, becomes computationally challenging because of repeated integration of the entire system of ODEs for large number of parameter samples. In order to reduce the computational burden, we constructed a RS-HDMR meta-model that replaces the selected output of ODEs with a hierarchy of polynomial functions. As outlined in Section 2.3.2, the resulting polynomial functions describe the contributions of the input parameters to the output, and can be directly related to the variance based Sobol' sensitivity indices. The workflow for the entire global analysis is presented in Figure 2. The original ODE model output generated for 10^5 samples of the 52 input parameters was used to train the meta-model. Subsequently, to test the performance of the meta-model, we randomly generated another parameter set different from the one used to train the model. The RS-HDMR model described the new set of data with an R^2 of 0.7884.

The Sobol' sensitivity indices of the 52 input parameters were then obtained using the procedure described in Section 2.3.3. All the first-order and second-order indices for this case summed to 0.7874, indicating that almost 79% of the total variance in the output can be explained by these indices. The prominent RS-HDMR based first- and second-order indices are shown by the solid bar graphs in Figures 3A and 3B, respectively. The parameters are ranked in the decreasing order of their sensitivity indices.

We further confirmed the accuracy of RS-HDMR estimates by comparing with direct MC evaluation, as shown by the black and patterned bars respectively in Figure 3A-B. The most sensitive parameter was k_{D6} with $S_i = 0.18$, representing the damage induced by proinflammatory action of IL-6. The next most sensitive parameter, k_6 , represents the rate parameter regulating production and degradation of IL-6 levels. Parameters k_{6M} , representing the production of IL-6 by macrophages, and k_{iNOSN} , representing neutrophil mediated iNOSd (iNOS) production rate were the next sensitive. The final set of parameters which make smaller contributions (but greater than 1%) are the damage recovery rate, k_D , initial LPS, activation of IL-6 by neutrophils, k_{6N} , and rate of regulation of IL-10 production and degradation, k_{10} . As shown in Figure 3B, the second-order indices have $S_{ij} < 0.025$, and are combinations of the important first-order parameters.

Collectively, many of the parameters that regulate IL-6 levels, for example IL-6 production and degradation and IL-6 production by inflammatory cells, affected the overall damage. At the same time, damage was also modulated by the iNOS production rate (particularly iNOS produced by neutrophils), the initial LPS levels, followed by IL-10 regulation rate. The importance of IL-6 and NO (through iNOS) on damage has also been identified in several experimental studies in the literature (Copeland et al, 2005; Kopf et al, 1994; Miki et al, 2005; Nathan, 1997; Wright et al, 1992; Zamora et al., 2000).

3.4 Interplay between damage and the sensitive parameters predicted by second-order RS-HDMR component functions

While global sensitivity analysis can identify the significant parameters, this methodology does not give information regarding the functional effect of a given parameter on the output of interest. The functional effect is very well represented by the RS-HDMR component functions (f_0, f_i and f_{ij}). We analyzed the first-order and second-order component functions for the first four most sensitive parameters (k_{D6} , k_6 , k_{6M} and k_{iNOSN}) and their following combinations ((k_{D6}, k_6) , (k_{D6}, k_{6M}) and (k_{D6}, k_{iNOSN}) and (k_6, k_{iNOSN}) . These parameters contributed to a total of 43% of the variance. The remaining components represented very small individual contributions to the total variance (as discussed in Section 3.3). The individual first-order component functions are presented in Figure A1. The combined effects of important first- and second-order functions for damage production are presented as a heat-map in Figure 4. The axes in the plots represent the pairs of the parameters x_i, x_j (normalized) and the heat value corresponds to their specific contribution to the output, i.e. $f_0 + f_i(x_i) + f_j(x_j) + f_{ij}(x_i, x_j)$, with the blue end of the spectrum indicating low AUC_D and red end of the spectrum indicating high AUC_D .

The effect of most sensitive parameter, k_{D6} , was reliant primarily on parameters k_6 , k_{6M} and k_{iNOSN} . As seen from Figure 4A, increasing values of k_{D6} increased damage (x-axis, shift

from blue to red), while increased negative regulation of IL-6 (k_6) reduced damage (y-axis, shift from red to blue when k_{D6} is high). Over the entire parameter space, there is a small window of parameter variations, at high k_{D6} and low k_6 that could result in high damage. This is consistent with Figure 1A, in which we observe that for most of the samples, damage tends to be low. Figure 4B shows the contribution of IL-6 production by macrophages. At low values of k_{6M} , k_{D6} fails to induce damage, while at large values of k_{6M} damage is strongly induced by k_{D6} . This finding indicates the nonlinear contribution of macrophage induced IL-6 production towards total damage.

Another important contribution to damage is by k_{iNOSN} shown in Figure 4C. High k_{iNOSN} could reduce the damaging effects of k_{D6} . To check whether this effect is independent of IL-6 levels, the relation between k_6 and k_{iNOSN} was explored in Figure 4D. k_6 indicates how well the levels of IL-6 are controlled especially at later stages following LPS challenge. At low k_6 , the levels of IL-6 remain high at later stages. We found that $k_{iNOSN} > 0.5$ results in low damage nearly independently of k_6 . This effect presents a second possible avenue by which to reduce the damaging effects of IL-6. Namely, NO produced by iNOS may dampen the damaging effects of IL-6 even in the presence of high IL-6 levels (as gauged by k_6). Thus, our analysis suggested that the tissue-protective effect of iNOS could considerably reduce the direct harmful effects of IL-6-associated processes. Given this parametric dependence, we next investigated the general interrelationship among these three model outputs.

3.5 Interrelationships between Damage, IL-6, and NO in the global parameter space

In Section 3.4 we established that IL-6 was ranked first in the set of mediators affecting damage, followed by iNOS. Therefore, we assessed the relationship between damage and IL-6 in the presence or absence of iNOS, in order to map the interrelationship between these outputs. For this analysis, it was necessary to first determine the cumulative IL-6 levels following parameter perturbations using the area under the IL-6 curves (AUC_{IL6}) up to 28 h after LPS challenge. The resulting histogram of IL-6 is presented in Figure 5A.

3.5.1 Features of IL-6 distribution and respective dynamic profiles—The AUC_{il6} distribution showed a bi-modal distribution, with most samples centered near the lower peak and remaining set of samples centered near the peak between $AUC_{IL6} = 8-10$. Since the AUC_{IL6} is a measure of the dynamics, it is expected that the dynamics will show a progressive change from low to high AUC_{IL6} levels. To mine the distinct set of dynamic profiles, we performed k -means clustering on 10^5 time profiles of IL-6 simulated from the parameter set used for Figure 5A using two different distance measures. First, we performed a k -means clustering using a Euclidean distance measure to segregate the profiles on the basis of levels of IL-6. Each of the resulting k -clusters was re-clustered using the correlation distance in order to identify coherent dynamic profiles at each level of IL-6. The quality of the clusters was assessed by the silhouette coefficient, and we chose the number of clusters for which more than 90% of the members had a silhouette coefficient greater than 0.6. The k -means clustering segregated the data into three clusters based on the range of AUC_{IL6} and three minor clusters for each major cluster based on the correlation distance. The three major clusters had AUC_{IL6} in the following ranges: (a) Cluster 1 (AUC_{IL6} until 5) (b) Cluster 2

(AUC_{IL6} from 5 to 15) and (c) Cluster 3 (AUC_{IL6} above 15). The time profiles for each of the three major clusters and the minor clusters are plotted in Figure 5B-D. The blue curves show the actual dynamic profiles, while the red curve represents the centroid of the minor clusters.

3.5.1.1 Cluster 1: Cluster 1 (Figure 5B) contains low IL-6 levels and exhibits two major types of profiles. The profiles either peak early (~ 2 -3 h after LPS administration) with recovery towards baseline, or they show a sustained behavior but at very low levels. Therefore, in this region, the action of anti-inflammatory cytokine, IL-10 is sensitive enough to bring IL-6 levels down.

3.5.1.2 Cluster 2: Cluster 2 (Figure 5C) contains profiles that exhibited an early peak in IL-6 as in Cluster 1; however, IL-6 continued to remain high even at the later time points. Some profiles showed additional delayed activation. Cluster 2 profiles remain in the transition region from Cluster 1 dynamics to Cluster 3.

3.5.1.3 Cluster 3: Cluster 3 (Figure 5D) contained profiles showing a continuous increase and very high IL-6 levels, indicating that IL-6 is well above the baseline (zero when inflammation is resolved). Therefore, the anti-inflammatory processes responsible for bringing IL-6 levels down are not sensitive in this regime.

In the remaining sections, we used the above cluster-based segregation of AUC_{IL6} to evaluate the Damage, IL-6 relation. We also investigated sensitive parameters affecting AUC_{IL6} that confirmed the results presented in Section 3.4 (see Figure A2).

3.5.2 AUC_D and AUC_{IL6} relation under iNOS mediation—Figure 6A shows a schematic of the explored positive relationship between IL-6, damage, and the immune cells (N_A , M_A) in the presence of inhibition by iNOS. Figures 6B-C show the histograms of AUC_{IL6} and AUC_D respectively. Figure 6D shows the relationship between AUC_{IL6} and AUC_D in the entire analyzed parameter space. The histograms for AUC_{IL6} and AUC_D are oriented so that they align alongside the x- and y-axis, of Figure 6D. As presented in Section 3.5.1, AUC_{IL6} ranges from 0 to 40. This axis can be divided into three regions based on the clusters obtained in the Section 3.5.1: (a) Group A (AUC_{IL6} up to 5) (b) Group B (AUC_{IL6} from 5 to 15) and (c) Group C (AUC_{IL6} above 15). Group A contained 62% of the data. As seen from the clustering analysis (Figure 5B), this group also contained most of the profiles in which IL-6 resolved at the later stages. Hence, damage was expected to be low. Group B contained 24% of the data. The total damage was relatively high in this region. This appeared to be due to the continued expression of IL-6 and its direct tissue-mediated damage, which is not resolved by IL-10. Therefore, the threshold for IL-10 mediated inhibition of IL-6 was crossed in this region. Group C contained 14% of the data. Interestingly, this group also contained low damage, comparable to Group A. Based on our SA results; low damage in this region could be an indication of the opposing effect of iNOS and increased sensitivity to iNOS in this region.

3.5.3 AUC_D and AUC_{IL6} relation under iNOS inhibition—As seen from the second-order RS-HDMR for AUC_D , an important factor that silenced the effect of IL-6-mediated

damage parameters was the iNOS production term. We tested this hypothesis by re-evaluating AUC_D and AUC_{IL6} in the absence of an iNOS term. These plots are presented in Figure 7. Figure 7A shows a schematic of the explored positive relationship between IL-6, damage and the immune cells (N_A , M_A) in the absence of iNOS. Figures 7B-C show the histograms of AUC_{IL6} and AUC_D respectively. Figure 7D shows the relationship between AUC_{IL6} and AUC_D in the entire analyzed parameter space. The histograms for AUC_{IL6} and AUC_D are oriented so that they align alongside the x- and y-axis, of Figure 7D. The Groups A, B and C are marked in Figure 7D similar to Figure 6. As observed in Figure 7D, there is a slight shift in the AUC_{IL6} distribution to the right compared to the Figure 6D. More strikingly, Group C conditions exhibit higher damage in the absence of the damage recovery by iNOS. This finding indicates that tissue-protective effects of iNOS can rescue damage in this region. Otherwise, the continued $IL-6 \rightarrow \text{damage} \rightarrow IL-6$ feedback processes result in sustained damage.

3.5.4 Mechanisms underpinning Global Damage, IL-6 and iNOS relationship—

We found significant differences in the dynamics of active macrophages and neutrophils, depending on the production rate of iNOS and the levels of IL-6. To present these results, we chose the two sensitive parameters (k_6 and k_{iNOSN}) that affect AUC_D and represent the effects of IL-6 and iNOS (via NO) on AUC_D , respectively, based on the results of GSA. Figure 8 shows the dynamics of active macrophages, active neutrophils, iNOS, NO, IL-6 and damage. Three different values of each parameter were chosen for this representation, which include the high and low values in the interval as well as the nominal value. This gives rise to 9 possible cases. The color of the curve shows the rate of iNOS production: red (high), blue (nominal) and green (low). The format of the lines represents the levels of IL-6 towards the late phase: dashed (high), solid (nominal), dotted + dashed (low). When the levels of IL-6 return to their basal levels, all the represented mediators show recovery. However, when IL-6 levels are high (blue and green dashed lines), the outcome is heavily dependent on iNOS production rate by the neutrophils. For example, if iNOS production is low, we see a second peak in the active macrophages and the neutrophils. At the same time, IL-6 levels show continuous increase and damage remains very high. This is very similar to the case of multiple organ failure or severe organ damage, a scenario that is also associated with presence of both overly activated as well as exhausted macrophages and neutrophils (Pinsky, 2004). If iNOS production is increased in this situation (red dashed lines), there is a dramatic reduction in damage, and the second peak in macrophages and neutrophils is lost, although neutrophils still remain elevated. This state is also followed by reduction in IL-6 levels. Thus, iNOS production may benefit the individual in the situation in which IL-6 is still high. This may not be beneficial in the long term, since the cumulative effect of IL-6 accumulation after 28 h may still lead to gradual organ failure.

One significant finding is the hypersensitivity of damage to iNOS in the regions in which IL-6 is high. This explains the mechanisms resulting in the triangular relationship presented in Figures 6-7. It is also important to note that when IL-6 levels are low (high k_6), increasing iNOS production slightly increases IL-6 due to iNOS contribution to IL-6. In contrast, at high IL-6 levels, there is a reduction in IL-6 with increasing iNOS production. This phenomenon can again be explained by the decreased damage leading to lowering of pro-

inflammatory mediators. Thus, the positive feedback driven by damage-mediated maintenance of inflammation occurs via immune hyper-activation and concomitant increase in IL-6 activity. We found that there were no significant differences in TNF- α and IL-10 levels as a function of these parameter changes (data not shown). Figure 9 shows a summary of the processes affecting the outcome of inflammation.

4. Discussion

Several mechanistic studies, from our group as well as others, have represented acute inflammatory process by detailed complex non-linear ODEs (Daun et al., 2008a; Foteinou et al., 2009a; Vodovotz and An, 2009). Parametric study of these complex non-linear ODEs reveals critical information on the prime drivers of inflammation and the influence of parameter perturbations on the model outputs. Information from such an analysis is also useful in order to explain the variability in the outcome of similar experiments (Nieman et al., 2012), which can be attributed at least in part to differences in input parameters. However, for complex models, such a parametric study is computationally expensive due to the high dimensionality and the non-linearity of the input and output space. Therefore, a meta-modeling approach, which allows such analysis of large-scale models to be conducted in a computationally efficient manner, will be useful. In this paper, we have been able to examine such a high-dimensional model of acute inflammation thoroughly using RS-HDMR.

Our approach builds on a previously-developed, high-dimensional nonlinear ODE model of endotoxemia containing key inflammatory cytokines, reactive NO species, physiological parameters such as blood pressure, and an overall measure of well-being of the system (i.e. whole-animal inflammatory damage) (Chow et al., 2005; Torres et al., 2009). When carrying out GSA of large-scale systems, the primary interest is in identifying the relative importance of individual parameters or their importance ranking, along with the value of the sensitivity index. Accordingly, we sought to rank the impact of the mediators of acute inflammation on the global response of inflammation measured via cumulative tissue damage/dysfunction. To this end, we first analyzed the importance of the stimulus (initial LPS dose) under combinatorial variation of the rate parameters. Using RS-HDMR, we analyzed the output generated from the combinatorial variation to identify key parameters and their relative contributions to tissue damage.

Using this approach, the cytokine IL-6 was identified as a key indicator of tissue damage status, in agreement with numerous prior studies (Ivady et al., 2011; Kruttgen and Rose-John, 2012; Song and Kellum, 2005). We further clustered the dynamics of IL-6 in the selected parameter space. This allowed us to create a global picture of the relationship between IL-6 and damage under acute inflammation as well as modulation of this relation by a secondary mediator such as iNOS. Finally, we supplemented this interrelationship with several mechanisms identified during our combinatorial exploration of the parameter space that could recapitulate several clinical scenarios like a healthy response or a heightened inflammatory activity even in the absence of the initial stimulus. In this work, we also compared the accuracy of RS-HDMR indices with traditional MC based approaches for selected highly sensitive parameters and found RS-HDMR to give very close estimates. In a

recent work, we proved the acceptable performance of RS-HDMR by a full-length analysis of the accuracy and ranking of the indices for a high dimensional insulin-signaling pathway model (unpublished observations).

4.1 Influence of endotoxin dose on the physiology of inflammation

Typically, endotoxemia is studied experimentally by administering different levels of LPS and then measuring the cytokines and other inflammatory mediators such as NO reaction products responsible for influencing the outcome (overall damage). In mice, several studies have identified a range of endotoxin doses that show gradually decreasing survival ratios from LD₁₀ to LD₁₀₀ with increasing doses in the range of 10-50 mg/kg. One characteristic feature of animals that are on the verge of death is the high levels of inflammatory mediators in systemic circulation (Foteinou et al., 2009b; Nieman et al., 2012; Vincent and Abraham, 2006). At this juncture, the mathematical models predict an ever-increasing damage term which fails to resolve itself within 28 h (Nieman et al., 2012). This is in contrast to a healthy response, in which the initially-elevated circulating inflammatory mediators return to their basal levels, and, therefore, damage recovers (Chow et al., 2005; Nieman et al., 2012).

Our original model at the nominal values of the parameters was successful in explaining a lethal dose of inflammation as 12 mg/kg in C57/BL6 mice with persistent high levels of IL-6 and damage (Chow et al., 2005). In our current model, we extended the single parameter set to include many possible combinations of the parameters, and identified the smallest LPS dose that could still give rise to a signature of lethality, i.e. high damage. This was identified to be as low as 0.02 mg/kg. Such high damage at a low LPS dose, although not seen under nominal conditions, may indicate weaker regulatory structures leading to uncontrolled inflammation. Some possible experimental scenarios where lower than normal lethal dose could be harmful include aged mice (Kovacs et al., 2009; Plackett et al., 2004) and mice subjected to multiple lethal challenges (Krakauer et al., 2010). Many studies also indicate the possibility of other host-specific factors as equally important in driving a self-resolving vs. a self-maintaining response of inflammation (Clermont et al., 2004; Daun et al., 2008b; Foteinou et al., 2009b; Nathan, 2002). Our GSA supports this hypothesis, since we found many of the host-specific factors to be on par or more important than the initial LPS dose. Therefore, we were successful in identifying the importance of nonlinearity as well as the multi-component nature of the inflammation network which has repeatedly concurred in other medium scale-ODE models (Foteinou et al., 2009b), data driven models (Croft et al., 2012), Bayesian models (An et al., 2012), etc.

4.2 Global sensitivity analysis identifies the importance of IL-6 as a driver and biomarker of damage in endotoxemic mice

From the combinatorial analysis of the parameter space of our inflammation model, we identified mechanisms that could result in a damage that recovered vs. damage that was severe and could not recover over 28 h. Both of these are relevant experimental outcomes as observed in the studies mentioned before. From the complete analysis of the parameter space in this work, we identified IL-6 as a prime driver of endotoxin-induced damage in mice. IL-6 profiles which showed an initial peak by 2 h and a return to basal levels was associated with a healthy response in our previous experiments (Chow et al., 2005). In

contrast, IL-6 profiles which showed a significant rise followed by a plateau that did not return to the basal levels was particularly identified in non-survivors in an endotoxemia study in swine (Nieman et al., 2012). These non-survivors showed gradual systemic collapse like MODS with significant correlation in the IL-6 profiles to the outcome.

Interleukin-6 is a pleiotropic cytokine that plays significant roles in the pathophysiology of multiple diseases and experimental inflammatory challenges, including acute endotoxemia (Ivady et al., 2011; Song and Kellum, 2005; Vincent and Abraham, 2006). Interleukin-6 can drive both pro- and anti-inflammatory processes (Scheller et al., 2011). In the context of experimental endotoxemia, Krakauer *et al.* reported significant up-regulation of TNF- α , IL-1, IL-6 and MIP-2 at high LPS doses in BALB/c mice (Krakauer et al., 2010). This study identified key differences between surviving and non-surviving populations subjected to the same LPS dose of 4 mg/kg. Mediators such as IL-6 exhibited higher peaks in the non-surviving population as compared to the surviving population by 8 h, and IL-6 was sustained at high levels in non-survivors even at 21 h after LPS administration. In contrast, surviving animals exhibited decreasing IL-6 levels by 21 h.

The results of our RS-HDMR-based GSA further suggest a key role for IL-6. However, it is possible that this role of IL-6 may be modulated by other inflammatory mediators in other settings (e.g. hemorrhagic shock) (Mi et al., 2011). Since our ODE model incorporates the ability to simulate trauma and hemorrhagic shock in addition to endotoxemia, future analysis of the parameter sets defined by RS-HDMR may help in the identification of principal drivers of these other insults.

4.3 Additional mechanisms underpinning different outcomes of endotoxin administration in mice

Apart from IL-6, the inflammation network on which the model is based includes direct influences of NO on damage. While IL-6 directly induces damage through its pro-inflammatory activity, local production of NO by iNOS and eNOS can have tissue-protective effects, and thus reduce overall damage. In our analysis, we found that the primary role of NO is to attenuate the overall effect of IL-6 on damage. Our analysis also showed that the tissue protective effect of NO was primarily through iNOS rather than eNOS. Evidence in support of these findings was recently reported by Miki *et al.*, who showed that iNOS-deficient mice had lower survival 24 h following LPS administration (25% vs. 70% in wild type controls) (Miki et al., 2005).

At high IL-6 levels ($AUC_{IL6} > 10$), we found that the levels of macrophages and neutrophils were elevated and exhibited a second peak as compared to the baseline parameter set. This phenomenon was observed when iNOS levels were low; increases in iNOS could significantly reduce damage concomitantly with the loss of the secondary inflammatory cell peaks. Alterations of Th1 and Th2 responses have been observed in endotoxemic iNOS-deficient mice (MacMicking et al., 1995; Miki et al., 2005; Wei et al., 1995), and elevated Th1 cytokines led to persistent and uncontrolled inflammation in iNOS-deficient mice (Miki et al., 2005). Therefore, we hypothesize that hyper-activation of inflammatory cells as well as preponderance of the various cytokines occurs in the absence of iNOS.

4.4 Limitations of the study

In this study, we were focused primarily on evaluating the effect of LPS-induced inflammatory response. Hence, we have maintained the model's initial conditions at a baseline level with very little inflammation. Changes in the initial conditions may have important consequences. Overly-elevated baseline inflammation would simulate a diseased or aged state (Fayad et al., 2001; Namas et al., 2013) or preconditioning due to various treatments (Wada et al., 2005) or prior exposure to inflammatory stimuli (Day et al., 2006). Such an analysis would help in tailoring treatments based on the initial state of the patient. Our model has the capability for incorporating these changes and this will be addressed in future studies.

We recognize that our model includes a representative, but admittedly limited, subset of mediators. However, our parametric analysis indirectly captures variability commonly arising in biological systems due to the strength of the network structure. In future studies, with the inclusion of additional mediators, GSA may identify additional mechanisms that are key to inflammatory outcomes. Further, we note that our analysis was focused only on one inflammatory challenge, namely endotoxemia, even though our model includes the ability to simulate trauma as well as hemorrhagic shock. Likewise, to simulate true sepsis, the model would have to be expanded to include pathogen dynamics. Also, we would need to account for the multi-scale propagation of severe inflammation from cellular to the organ level in order to describe the phenomenon of MODS completely (An et al., 2012); future GSA of our two-compartment, pig-specific model (Nieman et al., 2012) could help address this question.

5. Conclusions

The application of a computationally efficient GSA technique to a large-scale, nonlinear model of acute inflammation in mice has enabled us to hypothesize a nonlinear impact of IL-6 and NO on inflammatory damage. Certain levels of pro-inflammatory response may be sufficient for the role of inflammation in fighting infection. Increased levels of pro-inflammatory mediators can result in hyper-activation of immune cells and concurrently, with self-sustaining damage, lead to a systemic spillover of inflammation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported under NIH grant [P50-GM-53789 to Y.V.] and NIH New Innovator Award [1DP2OD006491-01 to I.B.].

References

- An G, Nieman G, Vodovotz Y. Toward Computational Identification of Multiscale “Tipping Points” in Acute Inflammation and Multiple Organ Failure. *Annals of Biomedical Engineering*. 2012:1–11. [PubMed: 22012081]
- Barton RR. Metamodels for simulation input-output relations. *ACM*. 1992:289–299.
- Barton, RR. Simulation metamodels. Vol. 1. *IEEE*; 1998. p. 167-174.

- Beutler B, Cerami A. The Biology of Cachectin/Tnf - a Primary Mediator of the Host Response. *Annual Review of Immunology*. 1989; 7:625–655.10.1146/Annurev.Iy.07.040189.003205
- Beutler B, Rietschel ET. Innate immune sensing and its roots: the story of endotoxin. *Nat Rev Immunol*. 2003; 3:169–76.10.1038/nri1004 [PubMed: 12563300]
- Bogdan C, Vodovotz Y, Letterio J. TGF β and IL-10: inhibitory cytokines regulating immunity and the response to infection. *Novel Cytokine Inhibitors*. 2000:217–242.
- Chow CC, Clermont G, Kumar R, Lagoa C, Tawadrous Z, Gallo D, Betten B, Bartels J, Constantine G, Fink MP. The acute inflammatory response in diverse shock states. *Shock*. 2005; 24:74–84. [PubMed: 15988324]
- Chu Y, Hahn J. Generalization of a parameter set selection procedure based on orthogonal projections and the D optimality criterion. *AIChE Journal*. 2012; 58:2085–2096.
- Clermont G, Bartels J, Kumar R, Constantine G, Vodovotz Y, Chow C. In silico design of clinical trials: a method coming of age. *Crit Care Med*. 2004; 32:2061–70. [PubMed: 15483415]
- Copeland S, Warren HS, Lowry SF, Calvano SE, Remick D. Acute inflammatory response to endotoxin in mice and humans. *Clinical and diagnostic laboratory immunology*. 2005; 12:60–67. [PubMed: 15642986]
- Croft M, Duan W, Choi H, Eun SY, Madireddi S, Mehta A. TNF superfamily in inflammatory disease: translating basic insights. *Trends Immunol*. 2012; 33:144–52.10.1016/j.it.2011.10.004 [PubMed: 22169337]
- Daun S, Rubin J, Vodovotz Y, Clermont G. Equation-based models of dynamic biological systems. *J Crit Care*. 2008a; 23:585–94.10.1016/j.jcrc.2008.02.003 [PubMed: 19056027]
- Daun S, Rubin J, Vodovotz Y, Roy A, Parker R, Clermont G. An ensemble of models of the acute inflammatory response to bacterial lipopolysaccharide in rats: results from parameter space reduction. *Journal of theoretical biology*. 2008b; 253:843–853. [PubMed: 18550083]
- Davis MJ, Skodje RT, Tomlin AS. Global Sensitivity Analysis of Chemical-Kinetic Reaction Mechanisms: Construction and Deconstruction of the Probability Density Function. *Journal of Physical Chemistry A*. 2011; 115:1556.
- Day J, Rubin J, Vodovotz Y, Chow CC, Reynolds A, Clermont G. A reduced mathematical model of the acute inflammatory response II. Capturing scenarios of repeated endotoxin administration. *J Theor Biol*. 2006; 242:237–56.10.1016/j.jtbi.2006.02.015 [PubMed: 16616206]
- Fayad L, Keating MJ, Reuben JM, O'Brien S, Lee BN, Lerner S, Kurzrock R. Interleukin-6 and interleukin-10 levels in chronic lymphocytic leukemia: correlation with phenotypic characteristics and outcome. *Blood*. 2001; 97:256–63. [PubMed: 11133769]
- Feil, B.; Kucherenko, S.; Shah, N. Comparison of monte carlo and quasi monte carlo sampling methods in high dimensional model representation. *Advances in System Simulation, 2009. IEEE; SIMUL'09. First International Conference on. IEEE; 2009. p. 12-17.*
- Feng X, Hooshangi S, Chen D, Li G, Weiss R, Rabitz H. Optimizing genetic circuits by global sensitivity analysis. *Biophysical journal*. 2004; 87:2195–2202. [PubMed: 15454422]
- Foteinou P, Yang E, Androulakis I. Networks, biology and systems engineering: A case study in inflammation. *Computers & chemical engineering*. 2009a; 33:2028–2041. [PubMed: 20161495]
- Foteinou P, Calvano S, Lowry S, Androulakis I. Modeling endotoxin-induced systemic inflammation using an indirect response approach. *Mathematical biosciences*. 2009b; 217:27–42. [PubMed: 18840451]
- Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Current opinion in immunology*. 2001; 13:114–119. [PubMed: 11154927]
- Hahn J, Edgar TF. An improved method for nonlinear model reduction using balancing of empirical gramians. *Computers & chemical engineering*. 2002; 26:1379–1397. % @ 0098-1354.
- Hindmarsh, AC. ODEPACK, A Systematized Collection of ODE Solvers. Stepleman, RS., et al., editors. Vol. 1. North-Holland, Amsterdam: 1983. p. 55-64. *IMACS transactions on scientific computation* 1, 55-64
- Hofmann SR, Rösen-Wolff A, Tsokos GC, Hedrich CM. Biological properties and regulation of IL-10 related cytokines and their contribution to autoimmune disease and tissue injury. *Clinical Immunology*. 2012

- Ivady B, Beres BJ, Szabo D. Recent advances in sepsis research: novel biomarkers and therapeutic targets. *Curr Med Chem*. 2011; 18:3211–25. [PubMed: 21671855]
- Jayaraman, A.; Hahn, J. *Methods in bioengineering: systems analysis of biological networks*. Artech House Publishers; 2009.
- Kiparissides A, Kucherenko S, Mantalaris A, Pistikopoulos E. Global sensitivity analysis challenges in biological systems modeling. *Industrial & Engineering Chemistry Research*. 2009; 48:7168–7180.
- Kiparissides A, Koutinas M, Kontoravdi C, Mantalaris A, Pistikopoulos EN. ‘Closing the loop’ in biological systems modeling—From the *in silico* to the *in vitro*. *Automatica*. 2011; 47:1147–1155.
- Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bluethmann H, Köhler G. Impaired immune and acute-phase responses in interleukin-6-deficient mice. 1994
- Kovacs EJ, Palmer JL, Fortin CF, Fulop T Jr, Goldstein DR, Linton PJ. Aging and innate immunity in the mouse: impact of intrinsic and extrinsic factors. *Trends Immunol*. 2009; 30:319–24.10.1016/j.it.2009.03.012 [PubMed: 19541536]
- Krakauer T, Buckley MJ, Fisher D. Proinflammatory mediators of toxic shock and their correlation to lethality. *Mediators of inflammation* 2010. 2010
- Kruttgen A, Rose-John S. Interleukin-6 in sepsis and capillary leakage syndrome. *J Interferon Cytokine Res*. 2012; 32:60–5.10.1089/jir.2011.0062 [PubMed: 22136372]
- Kumar R, Clermont G, Vodovotz Y, Chow CC. The dynamics of acute inflammation. *Journal of theoretical biology*. 2004; 230:145–155. [PubMed: 15321710]
- Lagoa CE, Bartels J, Baratt A, Tseng G, Clermont G, Fink MP, Billiar TR, Vodovotz Y. The role of initial trauma in the host's response to injury and hemorrhage: insights from a correlation of mathematical simulations and hepatic transcriptomic analysis. *Shock*. 2006; 26:592–600. [PubMed: 17117135]
- Li G, Rabitz H. General formulation of HDMR component functions with independent and correlated variables. *Journal of Mathematical Chemistry*. 2012; 50:99–130.
- Li G, Rosenthal C, Rabitz H. High dimensional model representations. *The Journal of Physical Chemistry A*. 2001; 105:7765–7777.
- Li G, Wang SW, Rabitz H. Practical approaches to construct RS-HDMR component functions. *The Journal of Physical Chemistry A*. 2002a; 106:8721–8733.
- Li G, Wang SW, Rabitz H, Wang S, Jaffé P. Global uncertainty assessments by high dimensional model representations (HDMR). *Chemical Engineering Science*. 2002b; 57:4445–4460.
- Li G, Rabitz H, Yelvington PE, Oluwole OO, Bacon F, Kolb CE, Schoendorf J. Global sensitivity analysis for systems with independent and/or correlated inputs. *The Journal of Physical Chemistry A*. 2010; 114:6022–6032. [PubMed: 20420436]
- Lyakh L, Trinchieri G, Provezza L, Carra G, Gerosa F. Regulation of interleukin-12/interleukin-23 production and the T-helper 17 response in humans. *Immunol Rev*. 2008; 226:112–31.10.1111/j.1600-065X.2008.00700.x [PubMed: 19161420]
- MacMicking JD, Nathan C, Hom G, Chartrain N, Fletcher DS, Trumbauer M, Stevens K, Xie Q, Sokol K, Hutchinson N. Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. *Cell*. 1995; 81:641–650. [PubMed: 7538909]
- Marino S, Hogue IB, Ray CJ, Kirschner DE. A methodology for performing global uncertainty and sensitivity analysis in systems biology. *J Theor Biol*. 2008; 254:178–96.10.1016/j.jtbi.2008.04.011 [PubMed: 18572196]
- Mathew S, Sundararaj S, Mamiya H, Banerjee I. Regulatory interactions maintaining self-renewal of human embryonic stem cells as revealed through a systems analysis of PI3K/AKT pathway. *Bioinformatics Accepted*. 2014 In Press. 10.1093/bioinformatics/btu209
- Matzinger P. The danger model: a renewed sense of self. *Science Signalling*. 2002; 296:301.
- Medzhitov R. Origin and physiological roles of inflammation. *Nature*. 2008; 454:428–35.10.1038/nature07201 [PubMed: 18650913]
- Mi Q, Constantine G, Ziraldo C, Solovyev A, Torres A, Namas R, Bentley T, Billiar TR, Zamora R, Puyana JC, Vodovotz Y. A dynamic view of trauma/hemorrhage-induced inflammation in mice: principal drivers and networks. *PLoS One*. 2011; 6:e19424.10.1371/journal.pone.0019424 [PubMed: 21573002]

- Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clinical Science*. 2012; 122:143–159. [PubMed: 22029668]
- Miki S, Takeyama N, Tanaka T, Nakatani T. Immune dysfunction in endotoxemia: Role of nitric oxide produced by inducible nitric oxide synthase. *Critical care medicine*. 2005; 33:716–720. [PubMed: 15818094]
- Miller MA, Feng XJ, Li G, Rabitz HA. Identifying biological network structure, predicting network behavior, and classifying network state with high dimensional model representation (hdmr). *PloS one*. 2012; 7:e37664. [PubMed: 22723838]
- Namas RA, Bartels J, Hoffman R, Barclay D, Billiar TR, Zamora R, Vodovotz Y. Combined in silico, in vivo, and in vitro studies shed insights into the acute inflammatory response in middle-aged mice. *PLoS One*. 2013; 8:e67419.10.1371/journal.pone.0067419 [PubMed: 23844008]
- Nathan C. Inducible nitric oxide synthase: what difference does it make? *Journal of Clinical investigation*. 1997; 100:2417. [PubMed: 9366554]
- Nathan C. Points of control in inflammation. *Nature*. 2002; 420:846–52.10.1038/nature01320 [PubMed: 12490957]
- Neugebauer EAM, Willy C, Sauerland S. Complexity and non-linearity in shock research: reductionism or synthesis? *Shock*. 2001; 16:252–258. [PubMed: 11580105]
- Nieman G, Brown D, Sarkar J, Kubiak B, Ziraldo C, Dutta-Moscato J, Vieau C, Barclay D, Gatto L, Maier K. A two-compartment mathematical model of endotoxin-induced inflammatory and physiologic alterations in swine*. *Critical care medicine*. 2012; 40:1052. [PubMed: 22425816]
- Okino MS, Mavrovouniotis ML. Simplification of Mathematical Models of Chemical Reaction Systems. *Chem Rev*. 1998; 98:391–408. [PubMed: 11848905]
- Parker RS, Clermont G. Systems engineering medicine: engineering the inflammation response to infectious and traumatic challenges. *J R Soc Interface*. 2010; 7:989–1013.10.1098/rsif.2009.0517 [PubMed: 20147315]
- Parker SJ, Watkins PE. Experimental models of Gram negative sepsis. *British journal of surgery*. 2001; 88:22–30. [PubMed: 11136305]
- Pinsky MR. Dysregulation of the immune response in severe sepsis. *Am J Med Sci*. 2004; 328:220–9. [PubMed: 15486537]
- Plackett TP, Boehmer ED, Faunce DE, Kovacs EJ. Aging and innate immune cells. *J Leukoc Biol*. 2004; 76:291–9.10.1189/jlb.1103592 [PubMed: 15039467]
- Prince JM, Levy RM, Bartels J, Baratt A, Kane JM III, Lagoa C, Rubin J, Day J, Wei J, Fink MP. In silico and in vivo approach to elucidate the inflammatory complexity of CD14-deficient mice. *Molecular Medicine*. 2006; 12:88. [PubMed: 16953560]
- Purswani MU, Eckert SJ, Arora HK, Noel GJ. Effect of ciprofloxacin on lethal and sublethal challenge with endotoxin and on early cytokine responses in a murine in vivo model. *Journal of Antimicrobial Chemotherapy*. 2002; 50:51–58. [PubMed: 12096006]
- Reynolds A, Rubin J, Clermont G, Day J, Vodovotz Y, Bard Ermentrout G. A reduced mathematical model of the acute inflammatory response: I. Derivation of model and analysis of anti-inflammation. *Journal of theoretical biology*. 2006; 242:220–236. [PubMed: 16584750]
- Rice, RG.; Do, DD. *Applied mathematics and modeling for chemical engineers*. John Wiley & Sons; 2012.
- Romani L, Puccetti P, Bistoni F. Interleukin-12 in infectious diseases. *Clinical Microbiology Reviews*. 1997; 10:611–636. [PubMed: 9336665]
- Sahle S, Mendes P, Hoops S, Kummer U. A new strategy for assessing sensitivities in biochemical models. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*. 2008; 366:3619–3631.
- Saltelli A, Tarantola S, Chan KS. A quantitative model-independent method for global sensitivity analysis of model output. *Technometrics*. 1999; 41:39–56.
- Saltelli, A.; Ratto, M.; Andres, T.; Campolongo, F.; Cariboni, J.; Gatelli, D.; Saisana, M.; Tarantola, S. *Global sensitivity analysis: the primer*. Wiley-Interscience; 2008.
- Salvemini, D.; Billiar, TR.; Vodovotz, Y. *Nitric oxide and inflammation*. Birkhauser; 2001.

- Scheff JD, Calvano SE, Androulakis IP. Predicting critical transitions in a model of systemic inflammation. *J Theor Biol.* 2013; 338:9–15.10.1016/j.jtbi.2013.08.011 [PubMed: 23973206]
- Scheff JD, Mavroudis PD, Foteinou PT, An G, Calvano SE, Doyle J, Dick TE, Lowry SF, Vodovotz Y, Androulakis IP. A multiscale modeling approach to inflammation: A case study in human endotoxemia. *Journal of Computational Physics.* 2012
- Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and antiinflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta.* 2011; 1813:878–88.10.1016/j.bbamcr.2011.01.034 [PubMed: 21296109]
- Schilders, WHA.; Van der Vorst, HA.; Rommes, J. Model order reduction: theory, research aspects and applications. Springer; 2008.
- Sobol' IM. On quasi-monte carlo integrations. *Mathematics and computers in simulation.* 1998; 47:103–112.
- Sobol' IM. Global sensitivity indices for nonlinear mathematical models and their Monte Carlo estimates. *Mathematics and computers in simulation.* 2001; 55:271–280.
- Song M, Kellum JA. Interleukin-6. *Critical care medicine.* 2005; 33:S463. [PubMed: 16340422]
- Teukolski, SA.; Flannery, BP.; Press, WH.; Vetterling, WT. Numerical Recipes in FORTRAN-The Art of Scientific Computing. University Press; 1989.
- Torres A, Bentley T, Bartels J, Sarkar J, Barclay D, Namas R, Constantine G, Zamora R, Puyana JC, Vodovotz Y. Mathematical modeling of posthemorrhage inflammation in mice: studies using a novel, computer-controlled, closed-loop hemorrhage apparatus. *Shock.* 2009; 32:172–178. [PubMed: 19008782]
- Vincent JL, Abraham E. The last 100 years of sepsis. *American journal of respiratory and critical care medicine.* 2006; 173:256–263. [PubMed: 16239619]
- Vodovotz Y, An G. Systems biology and inflammation. *Systems Biology in Drug Discovery and Development. Methods and Protocols.* 2009:181–201.
- Vodovotz Y, Billiar TR. In silico modeling: methods and applications to trauma and sepsis. *Crit Care Med.* 2013; 41:2008–14.10.1097/CCM.0b013e31829a6eb4 [PubMed: 23863232]
- Vodovotz Y, Clermont G, Chow C, An G. Mathematical models of the acute inflammatory response. *Current opinion in critical care.* 2004; 10:383–390. [PubMed: 15385756]
- Vodovotz Y, Csete M, Bartels J, Chang S, An G. Translational systems biology of inflammation. *PLoS Comput Biol.* 2008; 4:e1000014.10.1371/journal.pcbi.1000014 [PubMed: 18437239]
- Vodovotz Y, Constantine G, Rubin J, Csete M, Voit EO, An G. Mechanistic simulations of inflammation: current state and future prospects. *Mathematical biosciences.* 2009; 217:1–10. [PubMed: 18835282]
- Vodovotz Y, Chow CC, Bartels J, Lagoa C, Prince JM, Levy RM, Kumar R, Day J, Rubin J, Constantine G. In silico models of acute inflammation in animals. *Shock.* 2006; 26:235–244. [PubMed: 16912648]
- Wada Y, Otu H, Wu S, Abid MR, Okada H, Libermann T, Kodama T, Shih SC, Minami T, Aird WC. Preconditioning of primary human endothelial cells with inflammatory mediators alters the “set point” of the cell. *The FASEB journal.* 2005; 19:1914–1916.
- Wakahara K, Kobayashi H, Yagyu T, Matsuzaki H, Kondo T, Kurita N, Sekino H, Inagaki K, Suzuki M, Kanayama N. Major articles and brief reports-bacteria-Bikunin Suppresses Lipopolysaccharide-Induced Lethality through Down-Regulation of Tumor Necrosis Factor- α and Interleukin-1 β in Macrophages. *Journal of Infectious Diseases.* 2005; 191:930–938. [PubMed: 15717269]
- Wei X, Charlesi IG, Smithi A, Xu D, Muller W. mice lacking inducible nitric oxide synthase. *Nature.* 1995; 375:1.
- Wright CE, Rees DD, Moncada S. Protective and pathological roles of nitric oxide in endotoxin shock. *Cardiovascular research.* 1992; 26:48–57. [PubMed: 1516112]
- Zamora R, Vodovotz Y, Billiar TR. Inducible nitric oxide synthase and inflammatory diseases. *Mol Med.* 2000; 6:347–73. [PubMed: 10952018]

Abbreviations

AUC_D	area under the damage curve
AUC_{IL6}	area under the interleukin-6 curve
DAMPS	damage associated molecular pattern molecules
eNOS	endothelial NO synthase
GSA	global sensitivity analysis
HS	hemorrhagic shock
iNOS	inducible NO synthase
IO	input-output
IL	Interleukin
LPS	Gram-negative bacterial lipopolysaccharide (endotoxin)
MODS	Multiple Organ Dysfunction Syndrome
NO	nitric oxide
ODE	ordinary differential equation
RS-HDMR	Random Sampling High Dimensional Model Representation
SA	sensitivity analysis
TNF-α	Tumor necrosis factor alpha

Highlights

1. The parametric space of an acute inflammation model was analyzed using global sensitivity analysis (GSA)
2. GSA suggested the importance of IL-6 and NO in affecting inflammatory damage
3. Increasing IL-6 leads to transition from low to high sustained damage
4. At high IL-6, NO produced by macrophages can still repress overall damage

Figure 1. Model prediction of cumulative damage in the explored parameter space

(A) Histogram of AUC_D till 28 hr for 10⁵ Monte Carlo realizations of the parametric space.

(B) AUC_D as a function of the initial LPS concentration. LPS values from 0.001 to 20 mg/kg is shown here. The scatter plot (blue) denotes the AUC_D for different combinations of parameters (MC simulations) while the red curve (nominal curve) shows the AUC_D under nominal values of the input parameters. The nominal curve predicts a lethal LPS dose of 9-12 mg/kg while exploration of the parametric space shows that even low doses of LPS above 0.02 mg/kg may be lethal by 28 hr and depends on other host specific factors. These values correspond to a lethal AUC_D around 250.

Figure 2. Workflow for entire global sensitivity analysis using RS-HDMR

We start with selection of the input parameters, $\Xi_1, \Xi_2, \dots, \Xi_k$. The original input vectors, $\Xi_1, \Xi_2, \dots, \Xi_k$, may lie in the interval $[a_i, b_i]$. For RS-HDMR, the input variables must be normalized in $[0,1]$, say $x_1, x_2, \dots, x_k$. Several MC samples of input parameter are generated and the ODE model is then integrated to obtain the dynamics of the variables, S , for every MC sample. The model variables are described in Table 1 and the ODEs (dS/dt) are described in Section 2.2. Using these results, the output of interest, Y (like AUC_D) is generated for each of the MC samples. The resulting input-output matrix is utilized to generate the component functions of RS-HDMR followed by the variance based Sobol' indices as described in the Section 2. Note that RS-HDMR is constructed for the normalized inputs, x_i .

Figure 3. RS-HDMR estimates of the Sobol' sensitivity indices for AUC_D till 28 hr

(A) First order Sobol' indices. The first 8 ranked indices are shown separately while the remaining indices are presented as a cumulative bar named as OTHERS. For comparison the black bars show the actual values of the indices from direct MC integration using 10^5 samples while the patterned bars show the RS-HDMR estimates. We see good agreement between the two values as well as accurate ranking. (B) Important Second order Sobol' indices. Together, all the first and second order indices sum to 0.7874. The 2nd order RS-HDMR on a new simulated test dataset gave an $R^2=0.7884$.

Figure 4. Global interactions between the sensitive parameters

(A-C) Interplay between IL-6 induced damage (k_{D6}) and one of parameters, (A) parameter regulating IL-6 production and degradation (k_6) (B) IL-6 activation by macrophages (k_{6M}) (C) iNOS production by neutrophils (k_{iNOSN}). (D) Interplay between IL-6 regulation (k_6) and the iNOS production rate (k_{iNOSN}). The x- and y- axes show the normalized values of the parameters corresponding to the actual ranges as given in Table A1. In this heatmap, blue end of the spectrum represents low values of AUC_D while red end of the spectrum represents high values of AUC_D. The numbers overlayed onto the figure in the grayscale print version represent the heat values.

Figure 5. Features of AUC_{IL6} till 28 hr

(A) Histogram of AUC_{IL6} distribution for the entire parameter space. The distribution shows bimodality. (B-D) k -clusters showing typical dynamics of IL-6 segregated by AUC_{IL6} values. The dynamics is separated into Euclidean distance based k -clusters (shaded regions in B-C) followed by segregation of each such major cluster into three correlation distance based clusters (red or solid lines). (B) shows resolving dynamics or a very delayed and sustained response at low levels, (C) shows a sudden rise followed by steady intermediate levels close to the peak and (D) shows similar dynamics as (C) but with mostly very high IL6 levels at later time points. Some members of this cluster show a continuously increasing trend indicating out-of-control inflammation.

Figure 6. Relationship between AUC_{IL6} and AUC_D under iNOS mediation

(A) Schematic showing the influence of iNOS on the long-range positive feedback between $IL-6 \rightarrow Damage \rightarrow IL-6$. The arrows indicate both direct and indirect effects. (B) Histogram of AUC_{IL6} distribution. (C) Histogram of AUC_D distribution. (D) Scatter plot showing the relationship between AUC_{IL6} and AUC_D . The model predicts a triangular relationship between AUC_{IL6} and AUC_D . An interesting observation is the low damage seen at high AUC_{IL6} . The values near the boxes indicate the % of 10^5 MC samples that belong to the charted group.

Figure 7. Relationship between AUC_{IL6} and AUC_D in the absence of recovery by iNOS
 (A) Schematic showing the long-range positive feedback between $IL-6 \rightarrow Damage \rightarrow IL-6$ in the absence of iNOS mediated inhibition. The arrows indicate both direct and indirect effects. (B) Histogram of AUC_{IL6} distribution. (C) Histogram of AUC_D distribution. (D) Scatter plot showing the relationship between AUC_{IL6} and AUC_D . Here, the iNOS term in the damage equation was removed, as the schematic in panel A shows. The scatter plot shows a continuously increasing relationship between AUC_{IL6} and AUC_D . The previously seen low damage (Figure 6) at high IL-6 levels was absent and we see a shift to higher damage in the AUC_D histogram.

Figure 8. Mechanisms responsible for low and high damage

ODE model outputs, M_A (A), N_A (B), iNOS (C), NO (D), IL-6 (E) and Damage (F). The outputs are simulated for 9 combinations of the sensitive rate parameters k_6 and k_{iNOSN} . Damage shows significant changes when the IL-6 levels and the rate of iNOS production by the neutrophils are modulated. Under low and nominal IL-6 levels, damage shows recovering dynamics. However, at high and sustained IL-6 levels, damage is very high and there is hyper activation of immune cells. On the other hand, damage is significantly reduced when iNOS production is increased.

Figure 9. Summary of the most sensitive processes affecting Total damage and IL-6 levels
 The black lines (or solid lines) indicate the processes affecting damage, the red lines (or dashed lines) indicate the processes affecting IL-6 and the blue lines (or dotted lines) indicate the processes affecting both damage and IL-6. Tissue damage is the ultimate marker of the inflammatory process and, as observed by global sensitivity analysis, many of the effects on damage are through the processes influencing the IL-6 node (here A) and the iNOS node (here B). The arrows closer to the node have the strongest influence. Other connections between the nodes may exist but are found to be less sensitive from the current work. Importantly, even after the IL-10 threshold for node A suppression is reached, node B is able to keep down the detrimental effects of continued IL-6 by affecting other processes hyper-activating NO levels.

Table 1
Dynamic variables of the mathematical model

Model component	Comment
<i>LPS</i>	Immunostimulant derived from gram-negative bacteria or administered exogenously
Resting macrophages (M_R)	Circulating monocyte or local macrophages that act as a cellular pool for activated macrophages
Active macrophages (M_A)	Activated macrophages triggered by LPS, TNF, IL-6 and tissue dysfunction. Activation is down-regulated by anti-inflammatory cytokines
Resting neutrophils (N_R)	Renewable pool of local and circulating neutrophils susceptible to activation
Active neutrophils (N_A)	Pool of local and circulating activated neutrophils triggered by LPS, TNF, IL-6 and tissue dysfunction
Constitutive nitric oxide synthase (<i>eNOS</i>)	Participates in blood pressure homeostasis. Activity is increased by anti-inflammatory cytokines and decreased by LPS and TNF.
Inducible nitric oxide synthase (<i>iNOSd</i> , <i>iNOS</i>)	Increased by LPS and TNF in activated neutrophils and macrophages. Decreased by anti-inflammatory cytokines. <i>iNOSd</i> is a precursor to <i>iNOS</i> .
NO_2^- / NO_3^- (NO_3)	Stable nitric oxide reaction products; related to the intensity of the local production of nitric oxide by <i>iNOS</i> and <i>eNOS</i>
<i>TNF</i>	A major early pro-inflammatory cytokine secreted mainly by activated macrophages and also by activated neutrophils
<i>IL-10</i>	An early anti-inflammatory cytokine whose synthesis is triggered by pro-inflammatory stimuli, antagonizes TNF and <i>iNOS</i> via TNF-dependent and TNF-independent pathways
<i>IL-6</i>	A pro-inflammatory cytokine with additional anti-inflammatory effects
Adrenergic inhibitory activity (<i>CA</i>)	Represents inhibitory activity of the adrenergic system on the production of TNF and IL-6
<i>IL-12</i>	Secreted by activated macrophages in the presence of TNF or IL-6. This cytokine limits the activity of IL-10
Blood Pressure (<i>B</i>)	Homeostasis depends on the <i>iNOS</i> and <i>eNOS</i> activity
Tissue Damage (<i>D</i>)	Overall systemic damage resulting from inflammation process