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Computational modeling of cytokine signaling in microglia

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Figure S1. Model behavior with and without delays. (A) Model outputs are shown for continuous LPS = 1000 application starting at t = 0. (B) Normalized outputs are shown along with experimental data used for parameter estimation. (C) Peak responses to a range of LPS doses, each applied for three days, are shown for each model species. Sharp deflections in the DDE traces are indicated by arrows.

Figure S2. First order parameter sensitivities support model robustness. (A) The TNFα response to LPS is shown above a plot of the first order sensitivity indices, with a corresponding time scale. Only two parameters showed sensitivities above 0.1. (B) The mean TNFα response averaged over 100,000 simulations is shown along with the corresponding 95% confidence band. This averaged waveform is qualitatively similar to the reference waveform shown in red.

Figure S3. Local sensitivity analysis supports model robustness. (A) The histogram shows counts of both maximal sensitivity indices and absolute peak minimal indices. Very few of these values exceed unity. (B) Mean sensitivity indices are shown along with corresponding 95% confidence intervals for all indices >1 (equations S12-14). (C) For each parameter with an absolute sensitivity exceeding unity, we plotted the TNFα response waveform, averaged over 1000 simulations, along with the associated 95% confidence band. These averaged responses match the reference model waveform shown in red.

Figure S4. Multiple distinct parameter sets underlie similar dynamic cytokine profiles. (A) Squared error sums (equations S3,4) for 19 parameter estimation runs with randomly initiated parameters. The best fits with the lowest errors in model fit to data are indicated by blue. Intermediate and worst fit errors are shown in cyan and magenta, respectively. Reference simulations (see main text Fig 1) are shown as black dashed lines. (B) Dynamic cytokine profiles for all estimated parameter sets, colored based on the error classification shown in panel A.

Figure S5. Multiple distinct parameter fits support robustness of model predictions. (A) Profiles for the inhibitory influences of IL-10 and TGFβ on TNFα (see main text Fig 4B). Each row corresponds to a fit with the sum of squared error indicated in the panel (sse). (B) Gain is shown for all fits under WT and TGFβ KO conditions (compare with main text Fig 3B). (C) Adaptation profiles, as a function of LPS stimulus amplitude, are shown under the following conditions: WT, IL-10 KO, and TGFβ KO. Arrows to the right of (A) indicate model variants with discrepancies with data. For row 2 (sse = 0.66), TGFβ KO did not consistently increase Gain. For row 7 (sse = 0.89), IL-10–mediated inhibition of TNFα did not precede that of TGFβ.

Figure S6. TGFβ and IL-10 provide temporally distinct feedback inhibition to TNFα with delays. Simulation results for the DDE model are shown for comparison with corresponding results for the ODE model (see main text Fig 4) (A) Relative waveforms of TNFα, TGFβ, and IL-10 are plotted for comparison. (B) Normalized TGFβ and IL-10 contributions to the TNFα activation rate equation show that IL-10-mediated inhibition of TNFα precedes that of TGFβ. (C) Area under the curve was computed as a function of time for the TGFβ and IL-10 inputs to TNFα shown in panel A. Normalized AUCs are shown in panel C. The AUC ratio trace represents the fractional contribution of IL-10 relative to TGFβ.

Figure S7. TNFα gradient with respect to initial TGFβ level. (A) The normalized TNFα gradient is plotted in the direction of the TGFβ initial condition for comparison with main text figure 5. In each plot, the y-axis is defined by the TGFβ initial condition range and the x-axis is defined by the IL-10 initial condition range. Each column corresponds to a different time point at which the gradients were computed and each row corresponds to a different value of the TNFα initial condition.

Figure S8. Direct Lyapunov exponent analysis. (A) Schematic to guide interpretation of the data. DLEs are shown in the space defined by the initial levels of TGFβ and IL-10. Such plots are shown in (B) for different initial TNFα levels (rows) and time points (columns). All simulations involved the continuous application of LPS = 1000 starting at t = 0. (C) DLE plot for TNFα_0 = 0.01 and t = 48 hrs is shown along with the corresponding plot of $\nabla TNF|_{IL10_0}$ (see main text Fig 4B). This analysis shows that the maximal network sensitivity corresponds to the negative TNFα gradient with respect to IL-10_0.

Figure S9. Adaptation with and without delays. All analyses related to adaptation (see main text figure 6) were carried out for both DDE and ODE models of WT, IL-10 KO, and TGFβ KO phenotypes. (A) Adaptation index for TNFα is shown. (B) Peak LPS-induced TNFα response. (C) TNFα response three days following the initiation of the LPS stimulus. (D) Time from stimulus initiation until the TNFα peak. (E) Scaled area under the curve (AUC divided by 1000) for the LPS-induced TNFα response.

Figure S10. IL-10 inhibition reduces TNFα and TGFβ. (A) All plots display the maximal TGFβ level within 48 hr following a 2 hr LPS pulse (LPS range: 10-1000). TGFβ is plotted against the TNFα expression peak for WT and IL-10 KO simulations. The positive relation between TGFβ and TNFα suggests that inhibition of TNFα by IL-10 indirectly controls TGFβ expression. (B) Similar findings were obtained for the relation between IL-10 and TNFα.

Experimental data used for parameter estimation

All data were from microglial cell cultures stimulated with 1 μg/mL LPS. The LPS stimulus was applied to different samples for different durations and the resulting cytokine expression measurements were represented as time series. These data were obtained from four papers from the same lab.

The LPS-stimulated temporal profiles of IL-1β and IL-6 were obtained from [1]. The authors used primary microglia from one day old mouse cortex. IL-1β and IL-6 were detected using bioassays. The assays entailed assessment of cell proliferation using conventional cell lines. Standard curves were generated using recombinant proteins. Protein concentrations of IL-1β and IL-6 at different LPS stimulus durations were estimated based on the standard curves. LPS-induced temporal expression profile data for TNFα and TGFβ were obtained from [2]. Cortical microglia were obtained from one day old mice. TNFα and TGFβ protein expression levels were quantified using bioassays as described above. IL-10 data were obtained from [3]. Cultured microglia were obtained from human fetal brain tissue. IL-10 protein expression was quantified based on a bioassay applied following a range of LPS durations. CCL5 data were from [4], in which human fetal microglia were used and CCL5 protein was quantified with an enzyme-linked immunosorbent assay.

OR gating model

The OR gating model was implemented based on the following equations:

$$\frac{dC_x}{dt} = K_x + k_{i,x} \sum_i \frac{C_i^{n_{ix}}}{C_i^{n_{ix}} + {K_{ix}}^{n_{ix}}} - \left(k_{j,x} \sum_j \frac{{K_{jx}}^{n_{jx}}}{C_j^{n_{jx}} + {K_{jx}}^{n_{jx}}} + \gamma_x \right) C_x \tag{S1}$$

$$K_x = k_x \left(\frac{LPS}{LPS + K_{LPS,x}} \right) \tag{S2}$$

where $C_x = C_x(t)$ is the expression of cytokine x (TNFα, IL-1β, IL-6, TGFβ, IL-10, or CCL5) that is produced upon activation by LPS according to equation S2. In equation S2, k_x is the activation rate constant and $K_{LPS,x}$ is the half-maximal activation constant for LPS-mediated C_x production. The activation of C_x depends on C_i according the a Hill function characterized by half-maximal activation constant K_{ix} and cooperativity coefficient n_{ix}. Similarly, inhibitory cytokine C_j reduces C_x production according to a decreasing sigmoidal function characterized by K_{jx} and n_{jx}. The respective rate constants for activation and inhibition are $k_{i,x}$ and $k_{j,x}$. The degradation of C_x was determined by rate constant γ_x.

In an attempt to fit this model to experimental data, we executed 10,000 parameter variations in a 100-fold range relative to the reference parameter set for the ODE model (main text equations 1 and 2, see below). We selected to best candidate parameter sets, based on least squared error, and attempted to fit the model using MATLAB's *fmincon* function. None of the resulting parameter sets gave fits that remotely resembled the dynamics of the experimental data. Further detail on our parameter estimation procedures is presented below.

Parameter estimation and model comparison

All experimental data from the literature were acquired from pdf files using ImageJ [5]. These data sets were normalized to the interval [0,1]. All $K_{1/2}$ terms were initiated to values in nM based on available data (see Tables 4-9). All n_H terms were initiated to one and all τ_d terms were initiated to one. Non-linear optimization functions were employed in MATLAB based on gradient descent (*fmincon*) as well as random search based optimization via simulated annealing (*simulannealbnd*). Manual adjustments were also implemented. We performed global sensitivity analysis (see main text Methods and "Sensitivity analyses" below) and manually tuned highly sensitive parameters. We applied simulated annealing to our final manually tuned parameter sets and *simulannealbnd* converged upon the initially supplied final parameter set (see "Parameter variation analyses" below).

In general, parameter calibration with *fmincon* and *simulannealbnd* was accomplished by minimizing the sum of squared error between a normalized data waveform and normalized simulation profile:

$$SSE_x = \sum_{i=1}^{n_t} (C_{x,ti} - \widehat{C}_{x,ti})^2 \tag{S3}$$

$$J = \min_{\theta} \sum_{x=1}^{N_C} SSE_x \tag{S4}$$

where SSE_x is the squared error for cytokine x where $C_{x,t}$ is the normalized experimentally measured concentration at time t, $\widehat{C}_{x,t}$ is the corresponding simulated value (also normalized), and n_t is the number of time points for which experimental measures were obtained ($ti = t1, t2, ..., tn_t$). For S4, J is the objective function that was minimized by adjusting parameter values in the vector θ and N_C is the number of cytokines in the model. The model fit was constrained such that all outputs were on the same order Parameter variation analyses of magnitude. The purpose of this constraint was to avoid achieving a profile fit by making certain profiles arbitrarily low or high.

Model comparisons were implemented by computing SSE_x for each cytokine x in a given model and the total SSE for that model. Supplementary table 2 displays values of SSE_x and *total SSE* computed for the following model variants (see main text): ordinary differential equations (ODEs) with a TGFβ autoregulation loop (ODE_L), delay differential equations (DDEs) with a TGFβ autoregulation loop (DDE_L), ODEs without a TGFβ autoregulation loop (ODE_{nL}), and DDEs without a TGFβ autoregulation loop (DDE_{nL}).

Additionally, we computed the corrected Akaike information criteria (AIC_c) as follows for each cytokine [6]:

$$AIC_x = n_{t,x}\, log\left(\frac{SSE_x}{n_{t,x}}\right) + 2\, n_p \tag{S5}$$

$$AIC_{c,x} = AIC_x + \frac{2\, n_p\, (n_p + 1)}{n_{t,x} - n_p - 1} \tag{S6}$$

$$total\ AIC_c = \sum_{x=1}^{N_C} AIC_{c,x} \tag{S7}$$

where $n_{t,x}$ is the number of experimental time points in the data set for cytokine x, n_p is the number of parameters in the model for which AIC is computed, SSE_x and N_C are as described above. Supplementary table 3 displays values of AIC_x and *total* AIC_c for the following model variants described above.

To compare model variants using out AIC metrics, we computed an Akaike weight (w_i) for each model [6, 7]:

$$\Delta_i = AIC_{c,i} - min(AIC_c) \tag{S8}$$

$$w_i = \frac{exp\left(-\frac{\Delta_i}{2}\right)}{\sum_{i=1}^{4} exp\left(-\frac{\Delta_i}{2}\right)} \tag{S9}$$

where $AIC_{c,i}$ is the *total* AIC_c for model i and $min(AIC_c)$ is the minimal *total* AIC_c across all models. Based on these calculations, when we computed w_i we obtained $w = 0.999$ for the DDE_L model, $w = 0.001$ for the ODE_L model, and $w = 0$ for the 'no TGFβ loop' models. This result confirms that the DDE model shows a better fit to the data relative to the ODE model. However, we showed that the ODE and DDE models produce similar results for functional analyses of our cytokine network (see supplementary figures S1,6,9). Hence, to highlight the differences between the ODE model employed for the figures in the main text and the 'no TGFβ loop' models, we recomputed w_i without the DDE_L model. In this case, $w > 0.999$ was obtained for the ODE_L model. The differences in model fits were negligible between the DDE and ODE models with TGFβ autoregulation in comparison to the model variants without TGFβ autoregulation.

In summary, the results from our model comparisons (Supplementary Tables 2,3) indicate the following order of model performance, based on consistent results from evaluations of SSE and AIC (best to worst): DDE_L, ODE_L, DDE_{nL}, and ODE_{nL}. While the DDE_L model showed the best performance, the ODE_L exhibited similar behavior in a range of functional contexts (Figs S1,6,9). In contrast, both models which included TGFβ positive feedback autoregulation loops showed substantially improved performance compared to corresponding models without such autoregulation of TGFβ.

Sensitivity analyses

Global analysis

We implemented global sensitivity analysis as in our previous work [8]. The theory and numerical implementation, described briefly below, is based on previous work [8–10]. In general, the output of a model C is a function of its parameters θ: $C = f(\theta_1, \theta_2, ..., \theta_k)$ for a model with k parameters. The function can be expanded to a high dimensional model representation (HDMR) as follows:

$$C = f(\theta) = f_0 + \sum_i f_i + \sum_i \sum_{j>i} f_{ij} + \ldots + f_{1,2,\ldots,k}$$

where $f_i = f_i(\theta_i)$ and $f_{ij} = f_{ij}(\theta_i, \theta_j)$. The terms in the HDMR are related to various statistical properties of C:

$$f_0 = E(C)$$

$$f_i = E(C|\theta_i) - f_0$$

$$f_{ij} = E(C|\theta_i, \theta_j) - (f_0 + f_i + f_j)$$

where $E(.)$ is the expectation of the argument and $E(C|\theta_i)$ is the expected value of C when θ_i is held fixed

and all other parameters ($\sim i$) are varied. Hence, $E(C|\theta_i)$ is often written as $E_{\theta\sim i}(C|\theta_i)$ to highlight the fact that parameters other than i (i.e., $\sim i$) are varied for the computation of $E(.|\theta_i) = E_{\sim\theta_i}(.|\theta_i)$. The term $E(C|\theta_i, \theta_j)$ is the the mean value of C when all parameter other than θ_i and θ_j are varied with θ_i and θ_j fixed ($i \neq j$). These terms are utilized in variance computations implicated in global sensitivity calculations:

$$V_i = V\big(f_i(\theta_i)\big) = V\big(E(C|\theta_i)\big)$$

$$V_{ij} = V\big(f_{ij}(\theta_i, \theta_j)\big) = V\big(E(C|\theta_i, \theta_j)\big) - (V_i + V_j)$$

where $V(.)$ is the variance of the argument and V_{ij} represents the joint effect of the parameter pair (θ_i, θ_j) interaction on the variance. Note that $V\big(E(C|\theta_i)\big)$ can be written as $V_{\theta_i}\big(E_{\theta\sim i}(C|\theta_i)\big)$ to indicate that both θ_i and $\theta_{\sim i}$ are varied. For each parameter θ_i, a range of θ_i values are chosen. For each θ_i value in the range, all other parameters ($\theta_{\sim i}$) are varied and expectation with a single fixed θ_i is obtained. Thus, the variance is taken over the set of $E(C|\theta_i)$ values computed for the range of θ_i values. The total unconditional variance can be decomposed from the variance components as follows:

$$V(C) = \sum_i V_i + \sum_i \sum_{j>i} V_{ij} + \ldots + V_{1,2,\ldots,k}$$

Then sensitivity components, including first and higher order terms, are computed by dividing both sides by $V(C)$:

$$1 = \sum_i S_i + \sum_i \sum_{j>i} S_{ij} + \ldots + S_{1,2,\ldots,k}$$

where the first-order sensitivity indices, attributed to the individual effect of every parameter, are computed as follows:

$$S_i = \frac{V\Big(E(C|\theta_i)\Big)}{V(C)} = \frac{V_i}{V(C)} \tag{S10}$$

Note that the total variance can also be decomposed as follows:

$$V(C) = V\big(E(C|\theta_i)\big) + E\big(V(C|\theta_i)\big) = V\big(E(C|\theta_{\sim i})\big) + E\big(V(C|\theta_{\sim i})\big)$$

Then the variance attributable to parameter θ_i, given complete knowledge of all other parameters ($\theta_{\sim i}$), is

$$V(C) - V\big(E(C|\theta_{\sim i})\big) = E\big(V(C|\theta_{\sim i})\big)$$

and the total sensitivity of C to θ_i, including all first and higher order interactions, is given by dividing this expression by $V(C)$:

$$S_{T_i} = \frac{E\Big(V(C|\theta_{\sim i})\Big)}{V(C)} = 1 - \frac{V\Big(E(C|\theta_{\sim i})\Big)}{V(C)} = \frac{V_i + \sum_{j\neq i} V_{ij} + \ldots + V_{1,2,\ldots,k}}{V(C)} \tag{S11}$$

Hence, our global sensitivity analysis entailed evaluating both the individual effects of θ_i in the absence (S_i, equation S10) and presence (S_{T_i}, equation S11) of interactions with all other parameters. In particular, while we estimated these individual parameter sensitivities, the computations involved variations of both θ_i and $\theta_{\sim i}$. Thus, these global sensitivity indices are not specific to particular values of any parameters. In

contrast, for local sensitivity analysis (see below), the local sensitivities are specific to particular reference parameter values.

In the following, we describe the procedures employed to implement our global sensitivity analysis [9]. The reader can consult [9] for theoretical details. In general, we performed a Monte-Carlo based procedure for computing sensitivity indices using simulations of the model for a range of parameter variations ($N =$ 100,000). Pseudo-random parameter sets were generated using the Sobol sequence [8]. We established two independent parameter matrices, $\mathbf{A}$ and $\mathbf{B}$, each with k columns (one for each parameter) and N rows. We next established a set of k matrices, $\mathbf{C}_1$, $\mathbf{C}_2$, ..., $\mathbf{C}_k$, where $\mathbf{C}_k$ was obtained by taking $\mathbf{A}$ and replacing its kth column with the corresponding k^{th} column of $\mathbf{B}$. We define $\mathrm{a}^{(j)}$ as the jth column of $\mathbf{A}$, $\mathrm{b}^{(k)}$ as the kth column of $\mathbf{B}$, and $\mathrm{c}_k^{(k)}$ as the kth column of $\mathbf{C}_k$. Then $\mathrm{c}_k^{(i)} = \mathrm{a}^{(i)}$ for every $i \neq k$ and $\mathrm{c}_k^{(i)} =$ $\mathrm{b}^{(i)}$ for $i = k$. We integrated the model for all of these parameter matrices, thereby generating $N(k+2)$ simulations. The corresponding simulation results for cytokine x are annotated as follows:

$$y_{x,\mathbf{A}} = f_x(\mathbf{A})$$

$$y_{x,\mathbf{B}} = f_x(\mathbf{B})$$

$$y_{x,\mathbf{C}_i} = f_x(\mathbf{C}_i)$$

where $f_x(\mathbf{A})$ is the output of the model for cytokine x with parameter matrix $\mathbf{A}$. The results of these simulations were used to numerically estimate the first order sensitivity indices:

$$S_i = \frac{y_{x,\mathbf{A}} \cdot y_{x,\mathbf{C}_i} - f_0^2}{y_{x,\mathbf{A}} \cdot y_{x,\mathbf{A}} - f_0^2}$$

where

$$y_{x,\mathbf{A}} \cdot y_{x,\mathbf{C}_i} = \frac{1}{N} \sum_{j=1}^{N} y_{x,\mathbf{A}}^{(j)} y_{x,\mathbf{C}_i}^{(j)}$$

$$y_{x,\mathbf{A}} \cdot y_{x,\mathbf{A}} = \frac{1}{N} \sum_{j=1}^{N} y_{x,\mathbf{A}}^{(j)} y_{x,\mathbf{A}}^{(j)}$$

and

$$f_0^2 = \left(\frac{1}{N} \sum_{j=1}^{N} y_{x,\mathbf{A}}^{(j)} \right)^2$$

Similarly, the total sensitivity indices were computed as

$$S_{T_i} = 1 - \frac{y_{x,\mathbf{B}} \cdot y_{x,\mathbf{C}_i} - f_0^2}{y_{x,\mathbf{A}} \cdot y_{x,\mathbf{A}} - f_0^2}$$

The results of examining first-order sensitivity indices (equation S10) showed that only two parameters accounted for more than 10% of the variance in the TNFα response to LPS. Those parameters were also identified in the evaluation of total sensitivity (compare figures 2 and S2). Next, we plotted the mean TNFα waveform, averaged over all 100,000 simulations used in the global analysis. While this averaged waveform differed quantitatively from the LPS response of the reference model, both responses were qualitatively similar, thus supporting the conclusion that our model generates a physiological TNFα response to LPS even if all parameters are varied simultaneously. This analysis supported the conclusion

that our model is robust to parameter variations.

Local analysis

For comparison with our global sensitivity analysis, we computed model sensitivity to single parameters based on isolated parameter variations. We varied each parameter 1000 times over a uniform 2-fold range relative to the reference value and computed estimates of parameter sensitivity across time for all cytokines. Simulations entailed application of LPS = 1000 at time t = 0, as in the global analysis. Sensitivity indices were computed as follows [12]:

$$S_{x,i}(t) = \frac{\theta_{i,ref}}{C_{x,ref}(t)} \left(\frac{\partial C_{x,i}(t)}{\partial \theta_i} \right) \quad \text{(S12)}$$

$$\partial C_{x,i}(t) = C_{x,i}(t) - C_{x,ref}(t) \quad \text{(S13)}$$

$$\partial \theta_i = \theta_i - \theta_{i,ref} \quad \text{(S14)}$$

where θ_i is the the value of parameter i for a given sample $\theta_i \in (0.5\, \theta_{i,ref}, 2\, \theta_{i,ref})$, $\theta_{i,ref}$ is the value of parameter i obtained from our model calibration, $C_{x,ref}(t)$ is the simulated concentration of cytokine x at time t with model parameter set $\theta_{i,ref}$, and $C_{x,i}(t)$ is the $C_x(t)$ value computed with θ_i. We computed $S_{TNF\alpha}(t)$ for all parameters and found that only eight parameters were associated with maximal absolute sensitivities above unity (Fig S3). We computed the mean $S_{TNF\alpha}$ values, averaged over θ_i, along with associated 95% confidence intervals (Fig S3B):

$$\overline{S_{TNF\alpha}(t)} = \left(\frac{1}{N} \right) \sum_{\forall \theta_i} S_{TNF\alpha,i}(t) \quad \text{(S15)}$$

$$95\%CI = \overline{S_{TNF\alpha}(t)} \pm t_{0.025}(N-1) \frac{\widehat{\sigma}}{\sqrt{N}} \quad \text{(S16)}$$

where $N = 1000$ and $t_i(df)$ is the value of the t random variable with df degrees of freedom and tail probability 0.025, and $\widehat{\sigma}$ is the empirical standard deviation of $S_{TNF\alpha}(t)$. Our local sensitivity analysis identified all parameters found to be sensitive in the global analysis. This analysis showed that only eight of 88 total parameters (9%) have significant sensitivities to individual parameter variations over a 2-fold range [8]. Further, we evaluated TNFα responses to LPS for all parameters with absolute sensitivities above unity (Fig S3C). We plotted the mean TNFα response across 1000 simulations for each parameter, along with associated 95% confidence intervals. The results showed that the mean responses across 1000 simulations matched the behavior of the fitted model. These results independently support the global sensitivity analysis results, consistent with model robustness. The results show negligible variation are consistent with acceptable parameter uncertainty [13, 14].

Parameter variation analyses

Computational modeling of certain biological systems have shown that multiple parameter sets can be associated with similar, if not nearly identical, system behavior [15, 16]. We tested the hypothesis that multiple parameter sets can determine similar behavior of our cytokine network model. Our approach entailed randomly sampling 20 parameter sets using the Sobol sequence, where each parameter was varied within a two-fold range [8]. These 20 parameter sets were used as initial estimates for parameter estimation by simulated annealing with MATLAB's *simulannealbnd* function. In contrast to gradient

based optimization procedures, simulated annealing mitigates the risk for identifying local minima by randomly varying the parameter values such that a large region of parameter space is considered [17]. Simulated annealing has previously shown utility in calibrating S-system model parameters to experimental data [17]. The objective function we used for simulated annealing was the sum of squared error as described above ("Parameter estimation and model comparison").

Based on a wealth of research demonstrating that divergent parameter sets can generate consistent model output [18–20], along with research showing that many parameters in systems biology models exert negligible influences on model behavior [21], we expected that we would find multiple distinct parameter sets with comparable fits to experimental data. Our results from estimating parameters starting from 20 randomly sampled initial states supported our expectation. Out of 20 independent simulated annealing runs, 19 parameter estimates converged. We found that multiple distinct parameter sets provided data fits comparable to the fit of our reference model (Fig S4).

We further evaluated the model predictions associated with the parameter sets that gave the lowest sum of squared error (SSE) values (Fig S4, blue). These parameter sets had SSE values between 0.53 and 0.89 (Fig S5A). Note that the reference model had SSE = 0.58 and simulated annealing starting from the reference parameter set converged upon the reference parameter set. Hence, out of the 20 randomly chose initial parameter sets, only one fit resulted in lower SSE as compared to our reference parameter set. To evaluate the seven best fitted parameter sets, we first plotted the relative inhibitory input profiles for IL-10– and TGFβ–mediated inhibition of TNFα (Fig S5A). Only the fit with the highest SSE (= 0.89) did not show IL-10 input preceding that of TGFβ. Next, we tested whether the seven models exhibited TGFβ-sensitive tolerance to repeated LPS applications (see main text Fig 3 and associated text for details). Negative Gain was observed for all parameter sets, consistent with model validation of endotoxin tolerance. TGFβ KO increased Gain, thereby reducing tolerance, for 6/7 of the parameter sets (see row 2, SSE = 0.66; Fig S5B). Finally, we tested whether the effects of IL-10 KO and TGFβ KO observed for the reference model (main text Fig 6) could be obtained for the seven fitted models. In all cases, IL-10 KO increased adaptation relative to WT (Fig S5C), consistent with our experimental data (Fig 7). TGFβ KO decreased adaptation in all but two cases (SSE = 0.53 and SSE = 0.66). However, in both these cases, the effects of TGFβ were very small in comparison to the other simulations. In summary 5/7 parameter sets gave predictions regarding the temporal profiles of feedback inhibition to TNFα, tolerance and its dependence on TGFβ, and the effect of IL-10 KO on adaptation (see arrows to the left of A for discrepancies).

Parameter analysis discussion

Two distinct approaches exist for addressing the inverse problem of parameter estimation, which is often is ill posed such that multiple non-unique solutions exist [21, 22]. One general approach is to focus on model/parameter reduction [15,23], parameter identifiability assessment [24], and improved parameter estimation by utilizing regularization [25]. However, it has been demonstrated that unidentifiable parameters, characterized by exceedingly large or infinite confidence bounds, are ubiquitous in biological models [21] as well as models from physics [26]. Furthermore, it was shown that such parameter "sloppiness" is a fundamental property of physical systems that underlies the perceived independence of macroscopic macroscopic phenomena on microscopic parameters [26]. From the parameter sloppiness perspective, it was argued that focus should be directed to verifying the robustness of a model's dynamic profiles, which can be well constrained even if many of the underlying parameters are not [21]. For parameter values with low associated model sensitivities, it is correspondingly difficult to achieve parameter identifiability, even from extensive experimental sampling [21, 24]. Furthermore, even given high quality parameter measurements, the experimental conditions under which the measurements were obtained

my not foster generalizable estimates of the parameter values under physiologically relevant conditions [27].

Given that a spectrum of parameter sets can be associated with similar model predictions [21], it is informative to study populations of models characterized by distinct parameter sets that fall within a physiological range [15,28]. It is often assumed that one particular "true" parameter exists, but this notion is challenged by the large degree of molecular and physiological variability observed in experimental studies of single cells. Single cell molecular variability [29] suggests that a range of functional parameters are associated with homeostatic cellular function [20,30]. A wealth of experimental studies have demonstrated that divergent molecular state configurations are associated with qualitatively and quantitatively comparable physiological phenotypes [31]. Hence, parameter variability can be considered as a motivation rather than a hindrance for modeling. Furthermore, it has been shown that such variability can be functionally important [32,33]. Thus, our future efforts will explore the effects of parameter variability on microglial function.

Our parameter estimation necessarily underdetermined due to the large number of model parameters (93) in comparison to the number of data points (32). Furthermore, our model is set to arbitrary units of cytokine concentration because precise data are currently unavailable. Thus, our model can provide qualitative rather than quantitative predictions [22]. However, as detailed in"Sensitivity analyses", we applied both global and local sensitivity analyses and demonstrated that our model generates well constrained predictions. Consistent with our robustness analyses, we showed that multiple parameter states can generate similar model behavior (Fig S4,5). Despite the fact that multiple parameter sets determine similar behavior for our cytokine network model, the high degree of model robustness supports the generalizability of our model predictions [27]. Furthermore, our validation of the model based on (1) its replication of endotoxin tolerance phenomena and (2) its tolerance-dependence on TGFβ provides substantial evidence in favor of model validity.

Lyapunov exponent analysis

To systematically assess the sensitivity of TNFα, TGFβ and IL-10 LPS-mediated responses to the initial values of these cytokines, we performed a Lyapunov exponent analysis. We implemented the computation of direct Lyapunov exponents (DLEs) according to previously applied methods [34,35]:

$$DLE(t,\mathbf{x_0}) = log\left[\lambda_{max}\left(\left(\frac{\partial \mathbf{x}(t)}{\partial \mathbf{x_0}}\right)^T \left(\frac{\partial \mathbf{x}(t)}{\partial \mathbf{x_0}}\right)\right)\right] \tag{S18}$$

$$\mathbf{x} = [TNF\alpha \;\; TGF\beta \;\; IL10]^T$$

$$\frac{\partial \mathbf{x}(t)}{\partial \mathbf{x_0}} = \begin{bmatrix} \frac{\partial\, TNF\alpha(t)}{\partial\, TNF\alpha_0}\left(\frac{TNF\alpha_0}{TNF\alpha(t)}\right) & \frac{\partial\, TNF\alpha(t)}{\partial\, TGF\beta_0}\left(\frac{TGF\beta_0}{TNF\alpha(t)}\right) & \frac{\partial\, TNF\alpha(t)}{\partial\, IL10_0}\left(\frac{IL10\alpha_0}{TNF\alpha(t)}\right) \\ \frac{\partial\, TGF\beta(t)}{\partial\, TNF\alpha_0}\left(\frac{TNF\alpha_0}{TGF\beta(t)}\right) & \frac{\partial\, TGF\beta(t)}{\partial\, TGF\beta_0}\left(\frac{TGF\beta_0}{TGF\beta(t)}\right) & \frac{\partial\, TGF\beta(t)}{\partial\, IL10_0}\left(\frac{IL10_0}{TGF\beta(t)}\right) \\ \frac{\partial\, IL10(t)}{\partial\, TNF\alpha_0}\left(\frac{TNF\alpha_0}{IL10(t)}\right) & \frac{\partial\, IL10(t)}{\partial\, TGF\beta_0}\left(\frac{TGF\beta_0}{IL10(t)}\right) & \frac{\partial\, IL10(t)}{\partial\, IL10_0}\left(\frac{IL10_0}{IL10(t)}\right) \end{bmatrix} \tag{S19}$$

where x_0 is the concentration of cytokine x at time t = 0 and $\lambda_{max}(\mathbf{A})$ is the maximal eigenvalue of the matrix $\mathbf{A}$. The initial condition grid consisted of values between 0.01 to 20 varied in log space. DLEs were

computed over a range of time points and all simulations entailed continuous LPS = 1000 applications starting at t = 0 (see Fig S8).

Supplementary Tables

Note on Table 1: Network interactions characterized by cytokine activation and inhibition are represented by $\rightarrow$ and $\dashv$, respectively.

Note on Table 2: SSE values are shown for each cytokine in each model, along with the $totalSSE$s (equation S3). The model variants include: ordinary differential equations (ODEs) with a TGFβ autoregulation loop (ODE_L), delay differential equations (DDEs) with a TGFβ autoregulation loop (DDE_L), ODEs without a TGFβ autoregulation loop (ODE_{nL}), and DDEs without a TGFβ autoregulation loop (DDE_{nL}). Abbreviations: n_p = number of parameters, n_t = number of time points from the experimental data set.

Note on Table 3: AIC_c values are shown for each cytokine in each model, along with the $totalAIC_c$s (equations S5-7). Model annotation is as in Supplementary Table 2.

Note on Tables 4-9: k_{act} refers the the cytokine production rate constant in equation 1 of the main text (k_x), $K_{1/2}$ refers to a half maximal activation or inhibition constant (K_{ix} or K_{jx} in equation 1, respectively), n_H refers to the cooperativity coefficient (n_{ix} or n_{jx}), τ_d refers to the time delay term, and k_{deg} denotes the concentration-dependent degradation constant (γ_x).

Table 1. Network interactions

Interaction	Reference
LPS $\rightarrow$ IL-1β	[36]
TNFα $\rightarrow$ IL-1β	[36]
IL-6 $\dashv$ IL-1β	[37]
IL-10 $\dashv$ IL-1β	[38]
CCL5 $\dashv$ IL-1β	[39]
LPS $\rightarrow$ TNFα	[1]
IL-1β $\rightarrow$ TNFα	[2]
TNFα $\rightarrow$ TNFα	[40]
IL-6 $\dashv$ TNFα	[2]
TGFβ $\dashv$ TNFα	[2]
IL-10 $\dashv$ TNFα	[2]
CCL5 $\dashv$ TNFα	[39]
LPS $\rightarrow$ IL-6	[1]
TNFα $\rightarrow$ IL-6	[37]
IL-10 $\dashv$ IL-6	[41]
CCL5 $\dashv$ IL-6	[39]
TNFα $\rightarrow$ TGFβ	[2]
TGFβ $\rightarrow$ TGFβ	hypothesized
LPS $\rightarrow$ IL-10	[3]
TNFα $\rightarrow$ IL-10	[3]
IL-6 $\rightarrow$ IL-10	[3]
CCL5 $\dashv$ IL-10	[42]
LPS $\rightarrow$ CCL5	[4]
IL-1β $\rightarrow$ CCL5	[4]
TNFα $\rightarrow$ CCL5	[4]
IL-6 $\rightarrow$ CCL5	[4]
TGFβ $\dashv$ CCL5	[4]
IL-10 $\dashv$ CCL5	[4]

Table 2. Model comparisons: sum of squared error (SSE)

Model (n_p):	ODE_L (88)	DDE_L (94)	ODE_{nL} (86)	DDE_{nL} (92)
IL-1β ($n_t = 5$)	0.127	0.004	0.031	0.019
TNFα ($n_t = 7$)	0.347	0.157	1.365	0.55
IL-6 ($n_t = 5$)	0.037	0.039	0.020	0.043
TGFβ ($n_t = 7$)	0.010	0.038	0.256	0.291
IL-10 ($n_t = 4$)	0.011	0.014	0.051	0.020
CCL5 ($n_t = 6$)	0.051	0.018	0.241	0.322
total SSE	0.583	0.269	1.963	1.245

Table 3. Model comparisons: Akaike information criterion (AIC_c)

Model:	ODE_L	DDE_L	ODE_{nL}	DDE_{nL}
IL-1β	-20.8	-37.6	-27.9	-30.4
TNFα	-22.4	-27.9	-12.9	-19.1
IL-6	-26.9	-26.7	-30.0	-26.2
TGFβ	-47.6	-37.9	-24.6	-23.6
IL-10	-26.4	-25.4	-20.2	-23.9
CCL5	-30.6	-36.9	-21.3	-19.5
total AIC_c	-174.8	-192.5	-136.9	-142.8

Table 4. Parameter values: IL-1β regulation

Parameter	**Interaction**	**Value**	**Reference**
k_{act}	IL-1β production	1	estimation
$K_{1/2}$	LPS $\rightarrow$ IL-1β	3	[36]
τ_d	LPS $\rightarrow$ IL-1β	1	estimation
$K_{1/2}$	IL-1β $\rightarrow$ IL-1β	1	estimation
n_H	IL-1β $\rightarrow$ IL-1β	1	estimation
τ_d	IL-1β $\rightarrow$ IL-1β	1	estimation
$K_{1/2}$	TNFα $\rightarrow$ IL-1β	3.16	estimation
n_H	TNFα $\rightarrow$ IL-1β	1	estimation
τ_d	TNFα $\rightarrow$ IL-1β	5	estimation
$K_{1/2}$	IL-6 $\dashv$ IL-1β	5	[37]
n_H	IL-6 $\dashv$ IL-1β	1	estimation
τ_d	IL-6 $\dashv$ IL-1β	1	estimation
$K_{1/2}$	IL-10 $\dashv$ IL-1β	20	estimation
n_H	IL-10 $\dashv$ IL-1β	1	estimation
τ_d	IL-10 $\dashv$ IL-1β	1	estimation
$K_{1/2}$	CCL5 $\dashv$ IL-1β	900	[39]
n_H	CCL5 $\dashv$ IL-1β	1	estimation
τ_d	CCL5 $\dashv$ IL-1β	1	estimation
k_{deg}	IL-1β concentration-dependent degradation	0.01	estimation

Table 5. Parameter values: TNFα regulation

Parameter	Interaction	Value	Reference
k_{act}	TNFα production	900	estimation
$K_{1/2}$	LPS $\rightarrow$ TNFα	29	[1]
τ_d	LPS $\rightarrow$ TNFα	1	estimation
$K_{1/2}$	IL-1β $\rightarrow$ TNFα	1.61	[2]
n_H	IL-1β $\rightarrow$ TNFα	1	estimation
τ_d	IL-1β $\rightarrow$ TNFα	1	estimation
$K_{1/2}$	TNFα $\rightarrow$ TNFα	0.05	[40]
n_H	TNFα $\rightarrow$ TNFα	1	estimation
τ_d	TNFα $\rightarrow$ TNFα	1	estimation
$K_{1/2}$	IL-6 $\dashv$ TNFα	35.7	[2]
n_H	IL-6 $\dashv$ TNFα	1	estimation
τ_d	IL-6 $\dashv$ TNFα	1	estimation
$K_{1/2}$	TGFβ $\dashv$ TNFα	0.05	[2]
n_H	TGFβ $\dashv$ TNFα	1	estimation
τ_d	TGFβ $\dashv$ TNFα	1	estimation
$K_{1/2}$	IL-10 $\dashv$ TNFα	0.011	[2]
n_H	IL-10 $\dashv$ TNFα	1	estimation
τ_d	IL-10 $\dashv$ TNFα	1	estimation
$K_{1/2}$	CCL5 $\dashv$ TNFα	973	[39]
n_H	CCL5 $\dashv$ TNFα	1	estimation
τ_d	CCL5 $\dashv$ TNFα	1	estimation
k_{deg}	TNFα concentration-dependent degradation	0.1	estimation

Table 6. Parameter values: IL-6 regulation

Parameter	Interaction	Value	Reference
k_{act}	IL-6 production	0.4	estimation
$K_{1/2}$	LPS $\rightarrow$ IL-6	100	[1]
τ_d	LPS $\rightarrow$ IL-6	1	estimation
$K_{1/2}$	TNFα $\rightarrow$ IL-6	5	[37]
n_H	TNFα $\rightarrow$ IL-6	1	estimation
τ_d	TNFα $\rightarrow$ IL-6	1	estimation
$K_{1/2}$	IL-10 $\dashv$ IL-6	20	estimation
n_H	IL-10 $\dashv$ IL-6	1	estimation
τ_d	IL-10 $\dashv$ IL-6	1	estimation
$K_{1/2}$	CCL5 $\dashv$ IL-6	100	[39]
n_H	CCL5 $\dashv$ IL-6	1	estimation
τ_d	CCL5 $\dashv$ IL-6	1	estimation
k_{deg}	IL-6 concentration-dependent degradation	0.01	estimation

Table 7. Parameter values: TGFβ regulation

Parameter	Interaction	Value	Reference
k_{act}	TGFβ production	40	estimation
$K_{1/2}$	TNFα $\rightarrow$ TGFβ	55	[2]
n_H	TNFα $\rightarrow$ TGFβ	1	estimation
τ_d	TNFα $\rightarrow$ TGFβ	1	estimation
$K_{1/2}$	TGFβ $\rightarrow$ TGFβ	15	estimation
n_H	TGFβ $\rightarrow$ TGFβ	1	estimation
τ_d	TGFβ $\rightarrow$ TGFβ	1	estimation
k_{deg}	TGFβ concentration-dependent degradation	0.01	estimation

Table 8. Parameter values: IL-10 regulation

Parameter	Interaction	Value	Reference
k_{act}	IL-10 production	10	estimation
$K_{1/2}$	LPS $\rightarrow$ IL-10	100	[3]
τ_d	LPS $\rightarrow$ IL-10	1	estimation
$K_{1/2}$	TNFα $\rightarrow$ IL-10	22.5	[3]
n_H	TNFα $\rightarrow$ IL-10	0.5	estimation
τ_d	TNFα $\rightarrow$ IL-10	1	estimation
$K_{1/2}$	IL-6 $\rightarrow$ IL-10	20	[3]
n_H	IL-6 $\rightarrow$ IL-10	1	estimation
τ_d	IL-6 $\rightarrow$ IL-10	1	estimation
$K_{1/2}$	CCL5 $\dashv$ IL-10	30	[42]
n_H	CCL5 $\dashv$ IL-10	1	estimation
τ_d	CCL5 $\dashv$ IL-10	1	estimation
k_{deg}	IL-10 concentration-dependent degradation	0.01	estimation

Table 9. Parameter values: CCL5 regulation

Parameter	**Interaction**	**Value**	**Reference**
k_{act}	CCL5 production	3	estimation
$K_{1/2}$	LPS $\rightarrow$ CCL5	5	[4]
τ_d	LPS $\rightarrow$ CCL5	1	estimation
$K_{1/2}$	IL-1β $\rightarrow$ CCL5	1	[4]
n_H	IL-1β $\rightarrow$ CCL5	1	estimation
τ_d	IL-1β $\rightarrow$ CCL5	1	estimation
$K_{1/2}$	TNFα $\rightarrow$ CCL5	2	[4]
n_H	TNFα $\rightarrow$ CCL5	2	estimation
τ_d	TNFα $\rightarrow$ CCL5	1	estimation
$K_{1/2}$	IL-6 $\rightarrow$ CCL5	3	[4]
n_H	IL-6 $\rightarrow$ CCL5	1	estimation
τ_d	IL-6 $\rightarrow$ CCL5	1	estimation
$K_{1/2}$	TGFβ $\dashv$ CCL5	1	[4]
n_H	TGFβ $\dashv$ CCL5	2	estimation
τ_d	TGFβ $\dashv$ CCL5	1	estimation
$K_{1/2}$	IL-10 $\dashv$ CCL5	30	[4]
n_H	IL-10 $\dashv$ CCL5	1	estimation
τ_d	IL-10 $\dashv$ CCL5	1	estimation
k_{deg}	CCL5 concentration-dependent degradation	0.001	estimation

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