Competition and synergism between individual tumor necrosis factor-α activities determine granuloma structure during infection with *Mycobacterium tuberculosis*

Supplement 2: Detailed methods

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S2.1 Uncertainty and sensitivity analyses

With the Latin hypercube method we determine relevant ranges for each parameter (Tables 1 and 2), partition this range into M (= 250) intervals, and sample each interval once. We sample parameters from uniform or log-uniform distributions, depending on the size of the sampled parameter range (Table 1). These samples for each parameter are combined to form M total parameter sets.

Statistical sensitivity analysis allows the quantification of each uncertain parameter by correlating several outcome variables with variations in each parameter, to compute a partial rank correlation (PRC). One PRC exists for each parameter-variable pair, varying between –1 and 1, and representing the strength of relationship between the parameter and variable. We use a T test to determine if the correlations are significantly greater than zero, and a Z test to determine if two correlations significantly differ from each other. Due to the number of comparisons made, we use a false-detection correction method (FDR) to prevent spurious indications of significance. For a review of uncertainty and sensitivity analysis methods in systems biology, see (1).

One requirement of statistical sensitivity analysis used here is monotonicity between each parameter-variable relationship. Aleatory uncertainty may cause the model to violate this requirement. Based on recent work in our group (1), we use a modified methodology where each sampled parameter set is run X times, with the average of the outcomes used for the sensitivity analysis. Here X is chosen to be 4, which is sufficient to reduce the level of uncertainty for this analysis. Thus the total number of simulation runs for each sensitivity analysis is $4M = 1000$.

S2.2 Measurement of granuloma size

One benefit of an ABM is that it has a spatial representation. To take advantage of this, we developed an algorithm to determine granuloma size for use as an outcome variable in sensitivity analysis. The process was made as simple as possible, with the goal being a quantitative measure of a spatial characteristic for sensitivity analysis. First, a graph of each granuloma at 200 days post-infection (c.f. Figure S1) was manually scored for a granuloma-like structure. Cases lacking a distinct mass or ring of macrophages were assigned a size of 0. For the remaining, we determined the granuloma size based on the median distance from the grid center (coordinate (50, 50)) of all macrophage types defined to be a part of the granuloma. To define the edge of a granuloma, a macrophage was counted as being in the granuloma if more than 6 other macrophages were in its Moore neighborhood.

S2.3 Simulated deletion and depletion of TNF activities

Five separate parameters were changed to test alterations in specific TNF activities. In a total TNF deletion/depletion, total TNF secretion (parameter s_{TNF}) was set to 0. We removed the effect of TNF-induced trans-endothelial migration by setting TNF-related recruitment parameters (r_{MTNF} and r_{TTNF}) to 0, and the effect of TNF-induced apoptosis activity was removed by setting the probability of TNF-induced apoptosis (p_{apopt}) to 0. We removed macrophage sensitivity to TNF by setting the sensitivity threshold (τ_{TNF}) to an unattainable level (10^6). To remove specific TNF-induced effects on macrophages, we introduced auxiliary parameters τ_{actTNF} representing the threshold for TNF-induced activation, $\tau_{secrTNF}$, representing the threshold for TNF-induced

cytokine/chemokine secretion, and $\tau_{apoptTNF}$, representing the threshold for TNF-induced apoptosis. We then set each threshold to an unattainable level (10^6).

S2 Reference

1. Marino, S., I. Hogue, C. Ray, and D. Kirschner. 2008. A Methodology For Performing Global Uncertainty And Sensitivity Analysis In Systems Biology. *J Theor Biol* 254:178-196.