Supplemental Materials: Depletion of TGF-β1 Increases Bacterial Clearance by Cytotoxic T Cells in a Tuberculosis Granuloma

Table S1. Parameters and parameter ranges used to generate baseline containment simulations

Table S2. Significant PRCC values for TGF-β1 parameters introduced in this version of GranSim at day 200 PI

Figure S1. Decreased secretion of TGF-β1 by macrophages results in increasing percentage of effector cytotoxic T-cells at day 200.

Figure S2. Gating strategy for flow cytometry studies.

Table S1. Parameters and ranges used to generate baseline containment simulations

Parameter name	Value or range	Units
Mtb parameters		
Growth rate intracellular Mtb	1.003	cells
Growth rate extracellular Mtb	1.001	cells
Death rate of extracellular Mtb in caseum	1.5	cells
Core model parameters		
Diffusion time step	60	seconds
Molecular time step	6	seconds
Diffusion smoother time step	1.2	seconds
Number of smoother steps	0	n/a
Number of host cells causing caseation	10	n/a
Time to heal caseation	[1642, 2462]	days
Threshold for TNFα induced apoptosis	[1393, 2089]	molecules
Rate of TNFα induced apoptosis	[1.17e-6, 1.76e-6]	1/seconds
Minimum number of molecules allowing chemotaxis	[0.514, 0.77]	molecules
Maximum number of molecules allowing chemotaxis	[374, 562]	molecules

Diffusivity of TNFα	5.2e-8	cm^2/s
Diffusivity of IL10	5.2e-8	cm^2/s
Diffusivity of active TGF-β1	5.2e-8	cm^2/s
Diffusivity of chemokines	5.2e-8	cm^2/s
Degradation rate of TNFα	0.00158	molecules/molecular time step
Degradation rate of IL10	0.00048	molecules/molecular time step
Degradation rate of inactive TGF-β1	[9.28e-6, 1.39e-5]	molecules/molecular time step
Degradation rate of active TGF-β1	[8.0e-4, 0.0012]	molecules/molecular time step
Macrophage parameters		
Fraction of grid compartments with a macrophage	[0.024, 0.036]	n/a
Number of time steps before a resting macrophage can move	2	n/a
Number of time steps before an activated macrophage can move	16	n/a
Number of time steps before an infected macrophage can move	[112, 168]	n/a
Synthesis rate of TNFα ($MacTNF\alpha_{synth}$)	1.5	molecules/diffusion time step
Synthesis rate of CCL2	6	molecules/diffusion time step
Synthesis rate of CCL5	6	molecules/diffusion time step
Synthesis rate of CCL9	12	molecules/diffusion time step
Synthesis rate of IL10 by an activated macrophage	0.3	molecules/diffusion time step
Synthesis rate of IL10 by and infected macrophage	0.02	molecules/diffusion time step
Synthesis rate of Inactive TGF-β1 by macrophages	[1.4e-4, 2.12e-4]	molecules/diffusion time step

Number of bacteria a resting macrophage can phagocytose	1	n/a
Probability of resting macrophage killing bacteria ($MacKill_{baseline}$)	[0.23, 0.35]	n/a
Threshold for intracellular bacteria causing chronically infected macrophages	[8,12]	bacteria
Threshold for intracellular bacteria causing macrophage to burst	[13, 20]	bacteria
Number of bacteria an activated macrophage can phagocytose	[4, 6]	n/a
Fraction of inactive TGF-β1 activated by a mac ($Activation_{fraction}$)	[7e-5, 1e-4]	n/a
Amount of TGF-β1 that inhibits macrophages ($TGF\beta1max_{Mac}$)	[0.01, 1]	molecules
Fraction of active TGF-β1 in a compartment bound by a mac	[1.1e-5, 1.7e-5]	n/a
Probability of an activated macrophage healing a caseated compartment in its Moore neighborhood	[0.0128, 0.0129]	n/a
T cell parameters		
Probability of a T cell moving to the same compartment as a macrophage	[0.05, 0.08]	n/a
Probability of a T cell moving to the same compartment as a T cell	0.08	n/a
Synthesis rate of TNFα by IFNγ-producing T-cell	0.15	molecules/diffusion time step
IFNγ-producing T-cell probability of inducing Fas/FasL mediated apoptosis	[0.0152, 0.0228]	n/a

Probability of IFNγ-producing T-cell to secrete TNFα	[0.048, 0.072]	n/a
Probability of IFNγ-producing T-cell to secrete TNFα	[0.288, 0.432]	n/a
Synthesis rate of TNFα by cytotoxic T-cell	0.015	molecules/diffusion time step
Probability of a cytotoxic T-cell killing a macrophage	[0.012, 0.18]	n/a
Probability of a cytotoxic T-cell killing a macrophage and all associated Mtb	[0.61, 0.91]	n/a
Probability of cytotoxic T-cell to secrete TNFα	[0.056, 0.084]	n/a
Synthesis rate of IL10 by regulatory T-cell	0.739	molecules/diffusion time step
Synthesis rate of TGF-β1 by regulatory T cell	[0.0067, 0.0101]	molecules/diffusion time step
Probability a regulatory T cell will deactivate an activated macrophage	[0.011, 0.016]	n/a
Amount of TGF-β1 that inhibits T cells ($TGF\beta1max_{Tcell}$)	[0.01, 0.1]	molecules
Recruitment Parameters		
Maximum macrophage recruitment probability	[0.112, 0.168]	n/a
Maximum IFNγ-producing T cell recruitment probability	[0.112, 0.168]	n/a
Maximum cytotoxic T cell recruitment probability	[0.079, 0.12]	n/a
Maximum regulatory T cell recruitment probability	[0.0232, 0.0348]	n/a

* Indicates estimated parameters. All other parameters derived from prior work (70).

Table S2: Significant PRCC values for TGF-β1 parameters introduced to this version of *GranSim* at day 200 PI. p > 0.001

	Parameters

		Degradation Rate of Active TGF-β1	Degradation Rate of Inactive TGF-β1	Synthesis of Inactive TGF-β1 by Macs	Fraction of TGF-β1 Activated by Macs	Macrophage TGF-β1 Binding Rate	Maximum TGF-β1 Bound by a T cell	TGF-β1 Inhibition of Cytotoxic T cells	Synthesis of Inactive TGF-β1 by Regulatory T Cells
Outputs	# Total Macs							0.09	
	# Resting Macs							0.08	
	# Infected Macs		0.08					0.10	
	# Chronically Infected Macs								
	# Activated Macs		0.07					0.08	
	# Dead Macs				-0.09				
	# Total IFNg+ T cells							0.08	
	# Active IFNg+ T cells							0.08	
	# Down Regulated IFNg+ T cells								
	# Dead IFNg+ T cells								
	# Total Cytotoxic T cells							0.09	
	# Effector Cytotoxic T cells		0.09						
	# Total Regulatory T cells							0.07	
	# Intracellular Mtb		0.08					0.09	
	# Extracellular Mtb							0.09	
	# Non-replicating Extracellular Mtb							0.09	
	Total CFU		0.08					0.10	
	Total CEQ		0.09					0.16	
	# Mtb Killed by Apoptosis		0.09					0.17	
	# Mtb Killed by Cytotoxicity		0.08					0.13	
	# Mtb Killed by Fas/Fas-ligand		0.08					0.16	
	# Mtb Killed by Macs		0.09					0.13	
	# Mtb Killed in Caseation							0.14	
	Total TAG		0.08					0.14	
	Total TNF							0.08	
	Total CCL2							0.08	
	Total CCL5							0.10	
	Total CXCL9							0.10	
		Parameters							
		Degradation Rate of	Degradation Rate of	Synthesis of	Fraction of	Macrophage TGF-β1	Maximum TGF-β1	TGF-β1 Inhibition	Synthesis of Inactive

	Active TGF-β1	Inactive TGF-β1	Inactive TGF-β1 by Macs	TGF-β1 Activated by Macs	Binding Rate	Bound by a T cell	of Cytotoxic T cells	TGF-β1 by Regulatory T Cells
Outputs Total TAG							0.10	
Total Active TGFB			0.07				0.09	
Total Inactive TGFB		-0.28	0.23					0.15
Total TNFα Induced Mac Apoptosis		0.08					0.13	
TNFα Induced Resting Mac Apoptosis		0.07					0.11	
TNFα Induced Infected Mac Apoptosis		0.09					0.14	
Total Chronically Infected Mac Apoptosis							0.19	
TNFα Induced Activated Mac Apoptosis		0.07					0.12	
TNFα Induced T cell Apoptosis							0.11	
Fas/FasL Killing		0.08					0.15	
Cytotoxic Killing							0.12	
Total Chronically Infected Mac Bursting		0.10					0.15	
Total Pet Hot							0.08	

Macrophage TGFB binding rate, T cell TGFB binding rate had no significant correlations at day 200 PI*
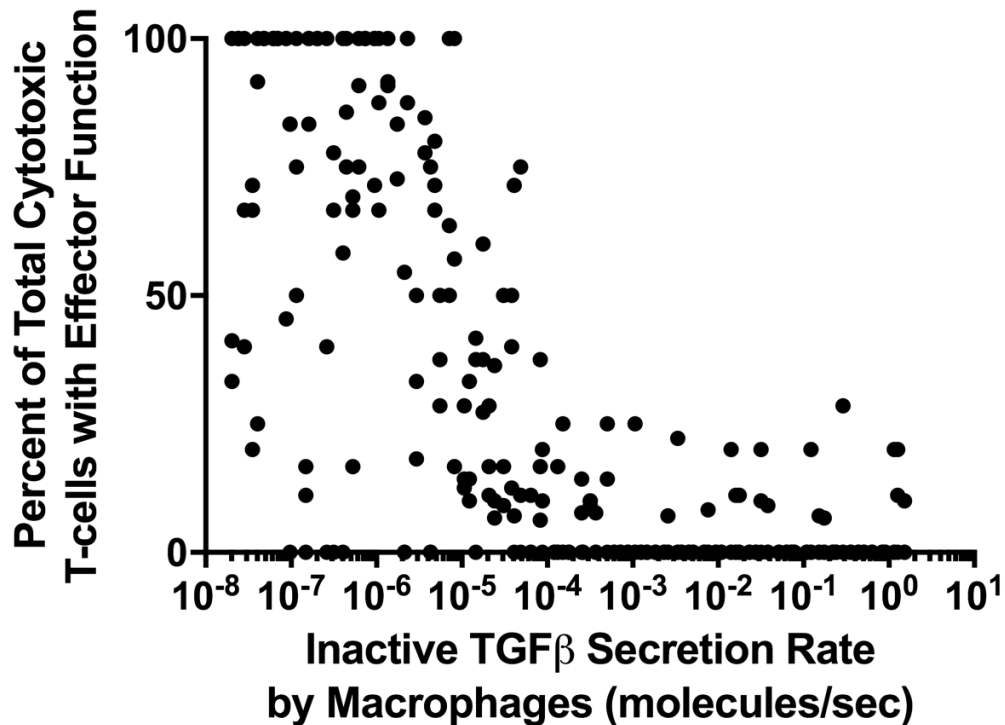
Figure S1: Simulated decreased secretion of TGFβ by macrophages results in increasing percentage of effector cytotoxic T-cells at day 200. 300 granulomas were simulated for 200 days with differing rates of latent TGFβ secretion by macrophages. The rate of secretion by macrophages is plotted against percent of total cytotoxic T-cells that are effector cytotoxic T-cells at day 200.

Effector cytotoxic T-cell activity shows sensitivity to different rates of TGF-β1 secretion.

Cytotoxic T-cell effector activity, and therefore bacterial killing efficiency, is inhibited by TGF-β1 signaling. In the absence of TGF-β1, effector cytotoxic T-cells in the granuloma are increased in number. Since macrophages are a major contributor to TGF-β1 levels in the granuloma, we compare how TGF-β1 secretion rates by macrophages affects the percent of effector cytotoxic T cells that are in granulomas (Fig. S1). We predict there is a negative correlation between the secretion rate of TGF-β1 and the percent of effector cytotoxic T cells in the granuloma (Fig. S1). In order to see a meaningful increase in the effector functions of cytotoxic T cells in our simulations, the TGF-β1 secretion rate by macrophages required a decrease by several orders of magnitude (Fig. S1).
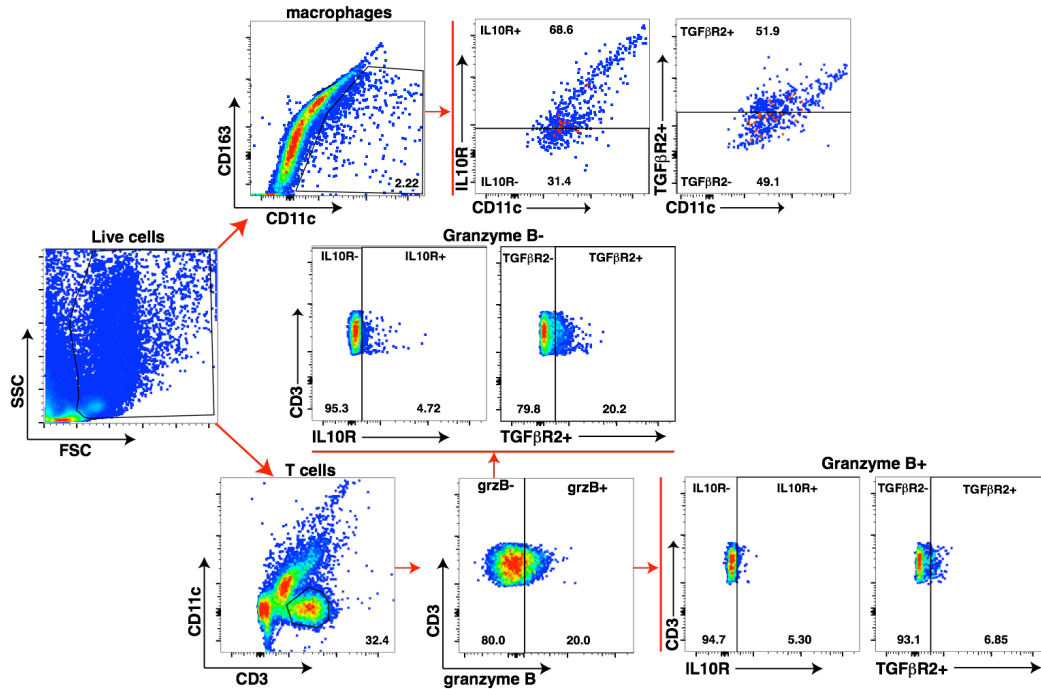
Figure S2: Gating strategy for flow cytometry studies. Granulomas do not contain enough cells for gating controls, and so positive and negative gates were determined by gating on erythrocyte-lysed whole blood (not shown) and these gates were then applied to granuloma samples. Isolated granuloma cells were gated on viable cells to exclude small, low complexity objects that confound analysis and then macrophages and T cells were identified by surface marker expression. Epithelioid macrophages and T cells were identified as CD11c+CD163- and CD3+CD11c- cells, respectively. T cells underwent a second round of gating against granzyme B to differentiate cytotoxic (granzyme B+) and noncytotoxic (granzyme B-) T cells. Subsequent analysis was done by gating each population's primary surface marker (CD11c or CD3) against IL10R or TGFBR2 expression, and comparing the MFI of positive and negative populations.