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

Supplement 1: Agent-Based Model Rules

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S1.1 Details of Agent-Based Model Rules

S1.1.1 Bacteria and effector molecules in the model

Chemokines and TNF are modeled by partial differential equations (PDEs) representing diffusion on the grid. Each molecular effector type (CCL2, CCL5, CXCL9/10/11 and TNF) is defined separately. We assume simple first-order diffusion with a term for signal degradation. Due to the scale difference between diffusing molecular signals and cells, we assume that diffusion is unaffected by the presence of cells, and concentrations of different types of molecules may be overlapping. Diffusion is solved in Δt = 6 second timesteps.

Numbers of Mtb are continuously represented as well. Since Mtb is a non-motile bacterium, we assume that bacteria do not diffuse, so we capture their numbers with discretized ordinary differential equations. In a given 20 μm^2 micro-compartment, bacteria grow according to a logistic growth law with a population capacity of K_{be} = 220: $B_e(t+1) = B_e(t) + \alpha_{be} B_e(t)(1 - B_e(t) / K_{be})$. The population limit of bacteria per micro-compartment is 10 times the number of Mtb contained within a macrophage before bursting. While the geometry of the micro-compartment allows up to approximately 450 individual bacilli in a single compartment due to the size of Mtb (a rod shape that is 2-5 μm long and 0.2-0.3 μm thick), we assume that there is a growth limitation due to competition for nutrient resources, preventing bacterial numbers from growing to this density.

S1.1.2 Environment

The 2-dimensional grid representation of lung tissue is a major simplification that makes spatial considerations computationally tractable. However, the representation requires some considerations to permit a realistic model of cellular crowding. Due to the size difference between macrophages and T cells, we allow up to two T cells to enter the same micro-compartment (with probability $4 \times T_{move}$), but only if no macrophage is present. A T cell may also move into the same micro-compartment as a macrophage (with probability T_{move}). This model of cell spacing is a compromise between a realistic spatial representation and computational tractability since we capture crowding effects while saving the computational cost of a continuous spatial representation. A three-dimensional approach has been developed (1) that confirmed results obtained with our previous two-dimensional model (2), suggesting that a two-dimensional approach is sufficient to capture first order effects.

In addition to movement and placement of cells on the grid, each micro-compartment also contains environmental variables that are affected by local conditions. These include the number of extracellular bacteria, the number of activated or infected macrophage deaths that occur in a micro-compartment, whether or not the micro-compartment has become caseous, and if that space is designated a vascular source.

We assume that a set number ($N_{caseum} = 6$) of deaths of activated or infected macrophages occurring in a micro-compartment causes the onset of caseation (this number can be varied in the analysis). When the last macrophage death leading to caseation is reached (i.e. N_{caseum} deaths have occurred), any T cell present in the micro-compartment is killed and no further cells are permitted entry to the micro-compartment.

If TNF and/or chemokine concentrations exceed a set threshold, then micro-compartments defined to be vascular sources have a chance of recruiting a macrophage or T cell at each timestep. The thresholds are set by parameters labeled *r* in Tables 1-2 (r_{MTNF}, r_{TTNF}, etc.).

S1.1.3 Rules for immune cells

Cells respond to local conditions according to rules that represent known activities *in vivo*. During simulations, each agent responds depending on its state. Several internal macrophage variables are set or altered by surrounding conditions: chemokine/TNF secretion (on/off), IFN-γ signal received (on/off), TNF or bacterial signal received (on/off), cell age, activation time, cell state (resting, activated, infected or chronically infected), and number of intracellular bacteria. Resting (M_r) and activated (M_a) macrophages can take up bacteria that are in the same micro-compartment. Resting macrophages may kill a small number of Mtb or become infected if the number of internalized bacteria exceeds 2. Production of chemokines and TNF by macrophages depends on activation by bacterial antigens and TNF; we therefore include a switch for chemokine secretion that is independent of the macrophage state (resting, infected, etc). If the macrophage detects sufficient TNF (above the threshold τ_{TNF}), it becomes capable of secreting TNF and chemokines. TNF and chemokine secretion is also induced by sufficient extracellular bacterial numbers in the same microcompartment ($B_{actM} = 100$). Infected macrophages secrete chemokines and TNF at half the rate in the absence of activation signals. Macrophages and T cells have a small probability of undergoing TNF-induced apoptosis (p_{apopt}) if the local TNF concenrtration exceeds τ_{TNF}.

During each update interval, infected macrophages may be activated by pro-inflammatory T cells (T_γ) in their *Moore neighborhood*, the 9-micro-compartment area around the cell location. Each pro-inflammatory T cell in the Moore neighborhood has a chance (T_{actm}) of activating an infected macrophage. For macrophages to reach bactericidal levels of macrophage activation, IFN-γ must work in concert with one other activation signal (either TNF or bacterial products) (3). Mtb-derived products only effectively complement IFN-γ in the model if extracellular bacterial levels at that location exceed a threshold ($B_{actM} = 100$). The contribution of T cell-derived IFN-γ to macrophage activation is represented with cell-cell interactions. This is an acceptable model since IFN-γ signaling requires close proximity of macrophage to T cell, as it is known to be secreted from T cells in a directed manner to the immunological synapse (4). Activated macrophages (M_a) effectively kill all their intracellular bacteria. A pro-inflammatory T cell (T_γ) that has successfully activated a macrophage then secretes TNF in a non-directed fashion (4) and becomes an IFN-γ secretor, so that the cell is able to activate the IFN-γ pathway in macrophages encountered thereafter.

If a macrophage is infected (M_i), intracellular Mtb divide at a rate set by parameter α_{Bi}. In the absence of activation, the intracellular number of Mtb may exceed a threshold (N_c, set to 10, half the carrying capacity of a macrophage, given below) where the cell becomes chronically infected (M_{ci}) after which it is incapable of being activated. Beyond a further threshold for intracellular bacterial numbers per macrophage (K_{bi}, set to 20 based on (5, 6)) the chronically infected macrophage bursts, releasing bacteria uniformly into the Moore neighborhood. This bursting, along with death of activated macrophages (M_a), contributes to caseation.

Cytotoxic T cells (T_c) randomly check one space in their Moore neighborhood each 10-minute time interval for the presence of infected macrophages. If an infected macrophage is present, the cytotoxic T cell has a low probability of killing that macrophage, along with all its intracellular bacteria if it was not chronically infected (reviewed in (7)). When chronically infected macrophages are killed, there is a 75% chance of all intracellular Mtb being killed, a 20% chance of dispersal to the Moore neighborhood, and a 5% chance of nothing occurring. T cell interactions with infected macrophages can also result in TNF-independent Fas/FasL-induced apoptosis (reviewed in (7)), resulting in 50% killing of intracellular Mtb and dispersal of the rest to the Moore neighborhood.

The mechanism of regulatory T cell (T_{reg}) function in Mtb infection is not well established, but may involve cell-contact mediated or immunosuppressive cytokine mechanisms (8). We adopt a cell-contact-mediated model of T_{reg} cell activity. T_{reg} cells check one space in the Moore neighborhood for the presence of a pro-inflammatory T (T_γ) cell. If it is present, cell-cell interaction occurs and the T_γ cell becomes incapable of activating infected macrophages for a set time afterwards ($t_{regT\gamma}$, 110 minutes by default). Since this time frame is not well established, we estimate it based on an approximate time to change the genetic program of the regulated cell while regaining activity.

S1.2 Outline of Rules

I. Initialization: conditions at the start of a simulation

a. 100 x 100 2-dimensional grid (representing 2 square mm)

i. Cellular boundary conditions:

periodic (toroidal)

ii. Molecular (chemokine and TNF) boundary conditions:

zero outside grid perimeter

b. 50 vascular source locations randomly distributed in 7 grid partitions

c. Microcompartment caseation counters set to 0

d. Distribute 105 resting macrophages randomly on grid

e. No chemokine or TNF present

f. 1 infected macrophage with 1 intracellular Mtb at the center of the grid

II. Overview: Timing and Order of Events

a. Diffusion/degradation of chemokine and TNF (if present) according to

$u_t = \lambda[\nabla^2 u] - \delta u$ for molecule u in Δt = 6 second increments (smallest timestep in model)[1]

b. Move macrophages based on CCL2/CCL5 (c.f. III.a.)

i. Move M_r on a 20-minute interval

ii. Move M_a on a ~13 hour interval

iii. Move M_i on a 24 hour interval

[1]Diffusion is solved in Δt = 6 second timesteps based on finding diffusion coefficients λ_c and λ_{TNF} from $\lambda = 4\delta\Delta t / \Delta x$, where δ is the molecular diffusion rate in m^2/s and Δx = 10^{-5} m^2 is the grid size (2. Segovia-Juarez, J. L., S. Ganguli, and D. Kirschner. 2004. Identifying control mechanisms of granuloma formation during M. tuberculosis infection using an agent-based model. *J Theor Biol* 231:357-376.

c. Events on 10-minute intervals

i. Move T_γ based on CCL2, CCL5, and CXCL9/10/11 (c.f. III.b.)

ii. Move T_c based on CCL5, CXCL9/10/11 (c.f. III.b.)

iii. Move T_{reg} based on CCL5 (c.f. III.b.)

iv. Determine macrophage (M_r) recruitment from each source:[2]

if r_{MTNF}*TNF(x,y) + r_{M2}*CCL2(x,y) + r_{M5}*CCL5(x,y) > r_M,

there is a probability M_{recr} of M_r recruitment

v. Determine T cell (T_γ, T_c and T_{reg}) recruitment:

1. Proportions are ρ_γ*$T_{\gamma recr}$ + ρ_c*T_{crecr} + ρ_r*T_{rrecr}

2. Pro-inflammatory T cell (T_γ) recruitment: if

$r_{T\gamma TNF}$*TNF(x,y) + $r_{T\gamma 2}$*CCL2(x,y) + $r_{T\gamma 5}$*CCL5(x,y) +

$r_{T\gamma 9}$*CXCL9/10/11(x,y) > $r_{T\gamma}$, ρ_γ = 1, otherwise ρ_γ = 0

3. Cytotoxic T cell (T_c) recruitment: if r_{TcTNF}*TNF(x,y) +

r_{Tc5}*CCL5(x,y) + r_{Tc9}*CXCL9/10/11(x,y) > r_{Tc}, ρ_c = 1,

otherwise ρ_c = 0

4. Regulatory T cell (T_{reg}) recruitment: if r_{TrTNF}*TNF(x,y) +

r_{Tr5}*CCL5(x,y) > r_{Tr}, ρ_r = 1, otherwise ρ_r = 0

vi. Determine cell-cell interactions, activation, chemokine production

1. M_r, M_i, M_{ci}, M_a (c.f. IV below)

2. T_γ, T_c, T_{reg} (c.f. V below)

[2] For the source at the microcompartment denoted by coordinates (x,y), TNF(x,y) represents the amount of TNF at that point; likewise for chemokines.

vii. Compute contribution of Mtb to chemotactic effects (Met-Leu-Phe, lipid antigens, etc.): modeled as contribution to CCL5 level

viii. Remove dead cells from the grid

ix. Calculate growth of extracellular Mtb according to

$$B_e(t+1) = B_e(t) + \alpha_{be} B_e(t)(1 - B_e(t)/(1.1 \cdot K_{be}))$$

d. Increment counter by 6 seconds, return to II.a.

III. Movement Rules

a. Each cell type has threshold (τ) and saturation (s) parameters for each chemokine it responds to.

b. Movement is random if all chemokines are below threshold or above saturation.

c. Macrophage chemotaxis:

i. Levels of CCL2 and CCL5 in surrounding microcompartments determine a probability distribution for movement

1. CCL2 affects movement if $\tau_{2m} < [\text{CCL2}] < s_{2m}$

2. CCL5 affects movement if $\tau_{5m} < [\text{CCL5}] < s_{5m}$

ii. Highest probability direction has further doubled probability

iii. Movement is blocked by

1. Caseous microcompartment

2. Macrophage presence

d. T cell chemotaxis:

i. Pro-inflammatory T cells (T_γ) depend on CCL2, CCL5 and CXCL9/10/11 (with saturation and detection thresholds as above)

ii. Cytotoxic T cells (T_c) depend on CCL5 and CXCL9/10/11 (with saturation and detection thresholds as above)

iii. Regulatory T cells (T_{reg}) depend on CCL5 (with saturation and detection thresholds as above)

iv. Movement is blocked/reduced by

1. Caseation (blocked)
2. Macrophage presence (probability of movement T_{moveM})
3. T cell presence (probability of movement T_{moveT})

IV. Rules for macrophages in each 10 minute interval

a. Resting (M_r):

i. Response to TNF: If local [TNF] exceeds a detection threshold (τ_{TNF}),

1. the cell becomes capable of secreting TNF and chemokines (CCL2, CCL5 and CCL9).
2. there is a chance (p_{apopt}) that TNF induces apoptosis of M_r cells.

ii. Phagocytosis of Mtb may result in infection:

1. If extracellular Mtb (b_e) $\leq N_{rk}$, the M_r kills them.
2. If $b_e > N_{rk}$:
    a. the M_r kills them with probability p_k
    b. the M_r becomes infected (M_i) otherwise

iii. Death due to age at a time uniformly distributed between 0 and 100 days after arrival on the grid.

b. Infected (M_i):

    i. TNF and chemokine secretion.

    ii. There is a chance (p_{apopt}) that TNF induces apoptosis.

        1. If this occurs, half of the intracellular bacteria survive and are distributed to the surrounding environment.

        2. Death contributes to the caseation counter at the location of the cell (microcompartment becomes caseous if the counter exceeds N_{caseum}).

    iii. Intracellular Mtb replicate according to $b_i(t) = b_i(t-1) + \alpha_{bi} b_i(t-1)$.

    iv. If intracellular Mtb number exceeds a threshold ($B_i > N_c$), the M_i becomes chronically infected (M_{ci}).

    v. Chance of activation by IFN-γ from pro-inflammatory T_γ cells not currently regulated by T_{reg}.

        1. With probability T_{actm}, any of the T cells may activate the macrophage; intracellular bacteria are killed and the cell becomes activated (M_a).

        2. The probability of activation saturates if the number of surrounding T_γ cells is above a certain number (N_{tact}).

    vi. If the M_i dies due to age, disperse intracellular Mtb into the Moore neighborhood surrounding the cell.

    vii. If the M_i dies due to age, increment the local caseation counter (the compartment becomes caseous if the counter exceeds N_{caseum}).

c. Chronically Infected (M_{ci}):

i. The cell undergoes the same secretion, apoptotic and bacterial growth events as infected macrophages (M_i), but is incapable of becoming activated.

ii. If the number of intracellular Mtb exceeds a threshold (K_{bi}),

1. The macrophage bursts
2. Intracellular bacteria are evenly distributed to the Moore neighborhood surrounding the M_{ci}.
3. Caseation counter is incremented

iii. The nominal lifespan is inherited from M_i predecessor

d. Activated (M_a):

i. Macrophages secrete chemokines and TNF

ii. Probability p_{apopt} of TNF-dependent apoptosis

iii. Actively take up and kill extracellular bacteria at a rate of N_{phag} bacteria per ten minute interval.

iv. M_a have a shortened lifespan of M_{als} (= 10 days) after activation

V. Rules for T cells in each 10 minute interval

a. Check for death due to age (uniformly distributed between 0 and 3 days after emergence from vascular source)

b. There is a chance (p_{apopt}) that TNF induces apoptosis.

c. Pro-inflammatory T_γ:

i. Chance of activating infected macrophages (M_i) via IFN-γ – detailed in section III.b.v.

ii. TNF secretion results from activation interaction

iii. Probability of TNF-independent induction of apoptosis in infected or chronically infected macrophages in surrounding compartments

a. Kill half of intracellular Mtb
b. Remaining Mtb uniformly distributed in Moore neighborhood
c. Increments local caseation counter

d. Cytotoxic T_c:

i. Chance of perforin/granulysin-mediated killing of M_i and M_{ci}

1. If M_i is found, chance of M_i and Mtb death, CCL5 release
2. If M_{ci} is found,
    a. 75% chance of M_{ci} and Mtb death, CCL5 release
    b. 20% chance of M_{ci} death, Mtb dispersal, CCL5 release
    c. 5% chance nothing happens
3. Probability of TNF-independent apoptosis induction in M_i or M_{ci}
    a. Kill half of intracellular Mtb
    b. Remaining Mtb uniformly distributed in Moore neighborhood
    c. Increment local caseation counter

e. Regulatory T_{reg}:

i. Chance of inactivating pro-inflammatory T cells (T_γ)

1. Inactive T_γ state lasts for $t_{regT\gamma}$ timesteps.

S1 References

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