Computational modeling of tuberculous meningitis reveals an important role for tumor necrosis factor-α

Supplementary Information

M. El-Kebir M. van der Kuip A.M. van Furth D.E. Kirschner

November 13, 2012

1 Supplementary Tables

Table 1: Containment parameter values obtained using LHS.

Parameter (unit)	**Description**	**Value**	**Ref**
Environment and general parameters			
D_{TNF} (cm^2/s)	Diffusion coefficient of TNF-α molecules	$5.2 \cdot 10^{-8}$	[1]
D_{CC} (cm^2/s)	Diffusion coefficient of chemokine molecules	$5.2 \cdot 10^{-8}$	[1]
δ_{TNF} (0.1min^{-1})	Degradation rate of TNF-α	0.0095	[2]
δ_{CC} (0.1min^{-1})	Degradation rate of the chemokines	0.0095	[2]
$\tau_{\text{TNF,apopt}}$	TNF-α level required for TNF-α-induced apoptosis	$[0.053, 0.073]$; 0.06	*
$p_{\text{TNF,apopt}}$	Probability by which TNF-α-induced apoptosis occurs	0.04	[3]
N_{sources}	The number of sources on the grid	200	[4]

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Table 1 – continued from previous page

Parameter (unit)	Description	Value	Ref
N_{caseous}	Number of killings for a micro-compartment to become caseous	$[5, 15]$; 7	*
Mycobacterium tuberculosis			
α_{Bi} (10min^{-1})	Growth rate of intracellular bacteria	0.0015	[5]
α_{Be} (10min^{-1})	Growth rate of extracellular bacteria	0.0007	[3]
K_{Be}	Number of extracellular bacteria a micro-compartment can accommodate	200	[3]
Phagocytes			
$s_{\text{P,TNF}}$ (#molecules/0.1min)	Maximal secretion rate of TNF-α by a phagocyte	$[0, 2.5]$; 0.74	*
$s_{\text{P,CCL2}}$ (#molecules/0.1min)	Maximal secretion rate of CCL2 molecules by a phagocyte	35	[3]
$s_{\text{P,CCL5}}$ (#molecules/0.1min)	Maximal secretion rate of CCL5 molecules by a phagocyte	35	[3]
$s_{\text{P,CXCL9}}$ (#molecules/0.1min)	Maximal secretion rate of CXCL9/10/11 molecules by a phagocyte	71	[3]
$\tau_{\text{P,TNF,NF}\kappa\text{B}}$	TNF-α level required for NFκB activation in phagocytes	$[0.01, 0.1]$; 0.014	*
$N_{\text{P,Be,NF}\kappa\text{B}}$	Number of extracellular bacteria in the Moore neighborhood required for NFκB activation in phagocytes	$[50, 500]$; 56	*
$N_{\text{P,Be,kill}}$	Number of extracellular bacteria that an active phagocyte can kill every time step	$[1, 3]$; 2	*

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Table 1 – continued from previous page

Parameter (unit)	Description	Value	Ref
$p_{\mathrm{P,STAT1}}$	Probability of a T_γ cell to enable STAT1 in a phagocyte	$[0.01, 0.1]$; 0.06	*
$v_{\mathrm{P,r}}$ (10min)	Time required for a resting phagocyte to move one micro-compartment	2	[6]
$v_{\mathrm{P,i}}$ (10min)	Time required for an infected phagocyte to move one micro-compartment	144	[3]
$v_{\mathrm{P,a}}$ (10min)	Time required for an active phagocyte to move one micro-compartment	$[36, 144]$; 134	*
$\sigma_{\mathrm{P,deact}}$ (10min)	Time span during which a phagocyte remains deactivated	$[36, 144]$; 124	*
N_{M}	Minimal number of meningeal macrophages on the grid	300	[3]
$N_{\mathrm{m,ram}}$	Minimal number of ramified microglia on the grid	500	[7]
$\tau_{\mathrm{M,recr}}$	Recruitment threshold for macrophage recruitment	$[0.02, 0.2]$; 0.06	*
$p_{\mathrm{M,recr}}$	Probability of recruiting a macrophage	$[0.01, 0.1]$; 0.05	*
$p_{\mathrm{P,kill}}$	Probability of a resting phagocyte to kill an extracellular bacterium	$[0.001, 0.1]$; 0.06	*
$\sigma_{\mathrm{m,prol}}$ (10min)	Number of timesteps a microglial cell is unable to proliferate following a proliferation event	$[144, 720]$; 605	*

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Table 1 – continued from previous page

Parameter (unit)	Description	Value	Ref
$N_{\mathrm{m,prol}}$	Maximum number of proliferation events a microglial cell can undergo	$[5, 15]$; 12	*
$\tau_{\mathrm{m,TNF,prol}}$	TNF-α/chemokine levels required for proliferation of reactive (either resting or active) microglia	$[0.5, 1.5]$; 1.4	*
$\tau_{\mathrm{m,TNF,react}}$	TNF-α/chemokine levels required for a ramified microglial cell to become resting	$[0.5, 1.5]$; 0.7	*
T cells			
$p_{\mathrm{Fas,apopt}}$	Probability of a T_γ cell to induce apoptosis via the Fas/FasL pathway	$[0.01, 0.1]$; 0.09	*
t_{recr} (10min)	Time after which T cell recruitment is enabled	$2,880$	[3]
$p_{\mathrm{T,P}}$	Probability of moving a T cell onto a micro-compartment containing a phagocyte	$[0.005, 0.05]$; 0.01	*
$p_{\mathrm{T,T}}$	Probability of moving a T cell onto a micro-compartment already containing a T cell	$[0.01, 0.1]$; 0.04	*
$\sigma_{\mathrm{c,deact}}$ (10min)	Time span during which a T_{cyt} cell remains deactivated	$[5, 50]; 30$	*
$\sigma_{\gamma,\mathrm{deact}}$ (10min)	Time span during which a T_γ cell remains deactivated	$[5, 50]; 19$	*
$p_{\mathrm{T,recr}}$	Probability of recruiting a T cell	$[0.05, 0.3]$; 0.27	*
$p_{\mathrm{c,kill}}$	Probability of a T_{cyt} cell to kill a (chronically) infected phagocyte	$[0.025, 0.25]$; 0.23	*

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Table 1 – continued from previous page

Parameter (unit)	Description	Value	Ref
$s_{\gamma,\mathrm{TNF}}$ (#molecules/0.1min)	Maximal secretion rate of TNF-α by a T_γ cell	$[0, 0.25]$; 0.074	*
$\tau_{\mathrm{T,recr}}$	Recruitment threshold for recruitment of T cells	$[0.1, 0.5]$; 0.4	*
$s_{\mathrm{c,TNF}}$ (#molecules/0.1min)	Maximal secretion rate of TNF-α by a T_{cyt} cell	$[0, 0.025]$; 0.0074	*
$p_{\mathrm{c,kill,cleanly}}$	Probability of a T_{cyt} cell to kill a chronically infected phagocyte cleanly	0.75	[2]

Table 2: p values assessing whether a pair of PRCCs differ significantly.

	$s_{\mathrm{P,TNF}}$	$p_{\mathrm{T,P}}$	$p_{\mathrm{T,recr}}$	$\tau_{\mathrm{P,TNF,NF}\kappa\mathrm{B}}$	$p_{\mathrm{M,recr}}$	N_{caseous}	$p_{\mathrm{T,T}}$
$s_{\mathrm{P,TNF}}$	-	0.0587	0.0000	0.0000	0.0000	0.0000	0.0000
$p_{\mathrm{T,P}}$		-	0.0000	0.0000	0.0000	0.0000	0.0000
$p_{\mathrm{T,recr}}$			-	0.0000	0.0000	0.0018	0.0006
$\tau_{\mathrm{P,TNF,NF}\kappa\mathrm{B}}$				-	0.2433	0.0000	0.0000
$p_{\mathrm{M,recr}}$					-	0.0000	0.0000
N_{caseous}						-	0.3733
$p_{\mathrm{T,T}}$							-

2 Supplementary Text

The ABM is run for 200 days by executing 28,800 10-minute time steps. The first step of the main loop concerns the secretion, diffusion and degradation of chemokines and cytokines. This happens at a finer timescale of 6-second time steps. Subsequently, all microglia are moved followed by moving macrophages and then T cells. After that microglial cells are proliferated and new immune cells are recruited. Then the immune cell specific rules are performed, and the growth function of extracellular Mtb is applied. The pseudo code of the main loop of the model is given in Algorithm 1.

Algorithm 1: BrainABM($\mathcal{G}, I$)

Input: $\mathcal{G}$ is a uniform grid and I is the initial inoculum

```
Initialize(G, I)
for t ← 1 to 28800 do
  for t' ← 1 to 100 do
    MacSecrete(G)
    MgSecrete(G)
    TcellSecrete(G)
    Diffuse(G)
    Degrade(G)
  MoveMicroglia(G, t)
  MoveMacrophages(G, t)
  MoveTcells(G)
  ProliferateMicroglia(G)
  Recruit(G, t)
  GrowExtMtb(G)
  UpdateStates(G, t)
```

Before starting the main loop, the grid is initialized by placing an initial inoculum on the grid. In our case the inoculum consists of one infected macrophage. This is followed by placing populations of $N_{\mathrm{m,ram}}$ ramified inactive microglia and N_{M} resting meningeal macrophages on the grid. The latter are placed at random on micro-compartments that are part of the meninges. The final step of the initialization is to place N_{sources} sources on the grid. This is done by subdividing the grid into $\lfloor\sqrt{N_{\mathrm{sources}}}\rfloor \times \lfloor\sqrt{N_{\mathrm{sources}}}\rfloor$ equally-sized clusters of micro-compartments; in each of these clusters a micro-compartment is picked at random and flagged to be a source. The remaining $N_{\mathrm{sources}} - \lfloor\sqrt{N_{\mathrm{sources}}}\rfloor \times \lfloor\sqrt{N_{\mathrm{sources}}}\rfloor$ sources are assigned in a random fashion by picking them from the micro-compartments that are not sources. In Algorithm 2 the pseudo code is given.

Algorithm 2: INITIALIZE($\mathcal{G}, I$)

Input: $\mathcal{G}$ is a uniform grid and I is the initial inoculum

`/* Initialize meninges */`

for $i \leftarrow 1$ **to** 100 **do**
  Let r be an integer, chosen uniformly at random, in the range $[10, 20]$
  for $j \leftarrow 100 - r$ **to** 100 **do**
    Let c be the micro-compartment at $\mathcal{G}_{ij}$
    c[meninges] $\leftarrow$ **true**

`/* Put inoculum and initial populations of macrophages and microglia on the grid */`

Place initial inoculum I on $\mathcal{G}$
Place N_{M} resting macrophages on random meningeal micro-compartments
Place $N_{\mathrm{m,ram}}$ ramified microglia on random micro-compartments of the grid

`/* Initialize sources */`

Subdivide $\mathcal{G}$ in $\lfloor\sqrt{N_{\mathrm{sources}}}\rfloor \times \lfloor\sqrt{N_{\mathrm{sources}}}\rfloor$ clusters of neighboring micro-compartments and in every such cluster, pick a micro-compartment c and set c[source] $\leftarrow$ **true**
Pick, uniformly at random, $N_{\mathrm{sources}} - \lfloor\sqrt{N_{\mathrm{sources}}}\rfloor \times \lfloor\sqrt{N_{\mathrm{sources}}}\rfloor$ micro-compartments that are not sources and for each such micro-compartment c set c[source] $\leftarrow$ **true**

2.1 Immune cell specific rules

Immune cell specific rules are applied in the function UPDATESTATES invoked in Algorithm 1. In this function we update the states of the immune cells on the grid, starting by macrophages followed by microglia, T_γ, T_{cyt} and finally T_{reg} cells. An immune cell is immediately removed from the grid, should it die during a state transition (e.g. due to age, or apoptosis). In order to prevent the order in which the various immune cell types are considered from playing a role, the state changes happen in a two-phase fashion: only after all immune cells are considered, the proposed new states are finalized (see Algorithm 3).

What all immune cells have in common are the attributes t_{birth} and t_{death} denoting, respectively, the birth and death time of an immune cell. The death time is determined during recruitment of the immune cell by taking the reported maximal lifespan into account. Microglia and macrophages have a maximal lifespan of 100 days [6], whereas effector T cells have a lifespan of at most 3 days [8].

In the remainder of this subsection we will describe the states and transitions of every immune cell. The death of a macrophage or a microglial cell may contribute to caseation in the micro-compartment in which it resides; once the number of killings contributing to caseation reaches N_{caseous}, the micro-compartment in question becomes caseous and any accompanying T cell in that micro-compartment is killed. Since this is a commonly used routine, we have isolated the pseudo code in Algorithm 4.

Algorithm 3: UpdateStates($\mathcal{G}, t$)

Input: $\mathcal{G}$ is a uniform grid and t is the current time step

Let $\mathcal{L}_{\mathrm{M}}$, $\mathcal{L}_{\mathrm{m}}$, $\mathcal{L}_{\gamma}$, $\mathcal{L}_{\mathrm{c}}$ and $\mathcal{L}_{\mathrm{reg}}$ be lists containing all macrophages, microglia, T_{γ} cells, T_{cyt} cells and T_{reg} cells, respectively
foreach $M \in \mathcal{L}_{\mathrm{M}}$ **do**
  UpdateMacState($M, \mathcal{G}, t$)
  if M[dead] **then** Remove M from $\mathcal{G}$
foreach $m \in \mathcal{L}_{\mathrm{m}}$ **do**
  UpdateMgState($m, \mathcal{G}, t$)
  if m[dead] **then** Remove m from $\mathcal{G}$
foreach $T_{\gamma} \in \mathcal{L}_{\gamma}$ **do**
  UpdateTgamState($T_{\gamma}, \mathcal{G}, t$)
  if T_{γ}[dead] **then** Remove T_{γ} from $\mathcal{G}$
foreach $T_{\mathrm{cyt}} \in \mathcal{L}_{\mathrm{c}}$ **do**
  UpdateTcytState($T_{\mathrm{cyt}}, \mathcal{G}, t$)
  if T_{cyt}[dead] **then** Remove T_{cyt} from $\mathcal{G}$
foreach $T_{\mathrm{reg}} \in \mathcal{L}_{\mathrm{reg}}$ **do**
  UpdateTregState($T_{\mathrm{reg}}, \mathcal{G}, t$)
  if T_{reg}[dead] **then** Remove T_{reg} from $\mathcal{G}$
Finalize all state transitions, by updating the current states of all cells

Algorithm 4: ContributeToCaseation(c)

Input: c is a micro-compartment

c[killings] $\leftarrow c$[killings] $+ 1$
if c[killings] $= N_{\mathrm{caseous}}$ **then**
  Kill any T cell in c
  c[caseous] $\leftarrow$ **true**

2.1.1 $\mathbf{T_{reg}}$ cell

In the model, a T_{reg} cell has only one state: it is always active. If a T_{reg} cell dies due to age or due to TNF-α-induced apoptosis, its death does not contribute to caseation. As seen in the pseudo code of Algorithm 5, a T_{reg} cell down-regulates every immune cell in its Moore neighborhood. If the immune cell to be down-regulated is a macrophage or a microglial cell then its transcription factor STAT1 is switched off. Note that T_{reg} cells themselves cannot be down-regulated.

Algorithm 5: UPDATETREGSTATE($T_{\text{reg}}, \mathcal{G}, t$)

Input: T_{reg} is T_{reg} cell, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which T_{reg} resides
if $t = T_{\text{reg}}[t_{\text{death}}]$ **then**
  $T_{\text{reg}}[\text{dead}] \leftarrow$ **true**
else if $c[\text{TNF}] > \tau_{\text{TNF,apopt}}$ **and** with probability $p_{\text{TNF,apopt}}$ **then**
  $T_{\text{reg}}[\text{dead}] \leftarrow$ **true**
else
  foreach immune cell ic in the Moore neighborhood of c that is not a T_{reg} cell **do**
    $ic[t_{\text{deact}}] \leftarrow t$
    $ic[\text{deact}] \leftarrow$ **true**
    if ic is a macrophage **or** ic is a microglial cell **then**
      $ic[\text{STAT1}] \leftarrow$ **false**

2.1.2 $\mathbf{T_{cyt}}$ cell

Just like other T cell types, the death of a T_{cyt} cell does not contribute to caseation. When a T_{cyt} is down-regulated by a T_{reg} cell, it does not perform its cytotoxic activities for a period of $\sigma_{\text{c,deact}}$ time steps. The cytotoxic activities of a T_{cyt} cell consist of killing infected or chronically infected phagocytes in its local micro-compartment. Whenever such a phagocyte is encountered, it is killed with a probability of $p_{\text{c,kill}}$. Killing a chronically infected phagocyte has two possible outcomes: either the phagocyte is killed cleanly and none of its intracellular bacteria are dispersed, or the killing proceeds in a less clean manner resulting in the dispersion of all intracellular bacteria over the Moore neighborhood. The probability of killing a chronically infected phagocyte in a clean manner is given by $p_{\text{c,kill,cleanly}}$. Cytotoxic killing of a macrophage contributes to caseation. In Algorithm 6 the pseudo code of this procedure is given.

2.1.3 $\mathbf{T_{\gamma}}$ cell

In addition to playing a crucial in the activation of phagocytes (by enabling the transcription factor STAT1 as we will see later on), T_{γ} cells have the ability to induce, with a probability of $p_{\text{Fas,apopt}}$,

Algorithm 6: UPDATETCYTSTATE($T_{\text{cyt}}, \mathcal{G}, t$)

Input: T_{cyt} is T_{cyt} cell, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which T_{cyt} resides
if $t = T_{\text{cyt}}[t_{\text{death}}]$ **then**
  $T_{\text{cyt}}[\text{dead}] \leftarrow$ **true**
else if $c[\text{TNF}] > \tau_{\text{TNF,apopt}}$ **and** with probability $p_{\text{TNF,apopt}}$ **then**
  $T_{\text{cyt}}[\text{dead}] \leftarrow$ **true**
else
  if $T_{\text{cyt}}[\text{deact}]$ **and** $T_{\text{cyt}}[t_{\text{deact}}] + \sigma_{\text{c,deact}} = t$ **then**
    $T_{\text{cyt}}[\text{deact}] \leftarrow$ **false**
  if $T_{\text{cyt}}[\text{deact}] =$ **false**
  and c has an infected or chronically infected phagocyte
  and with probability $p_{\text{c,kill}}$ **then**
    Let M be the phagocyte in c
    if $M[\text{state}] =$ `Chronically infected` **and** with probability $(1 - p_{\text{c,kill,cleanly}})$ **then**
      Disperse all intracellular bacteria over the Moore neighborhood of c
    $M[\text{dead}] \leftarrow$ **true**
    CONTRIBUTETOCASEATION(c)

so called Fas/FasL apoptosis in infected and chronically infected microglia and macrophages resulting in the dispersion of half of the intracellular bacteria over the Moore neighborhood. Unlike TNF-α-induced apoptosis, apoptosis due to the Fas/FasL pathway does contribute to caseation (see Algorithm 7). If a T_γ cell is down-regulated, it does not induce apoptosis for a period specified by $\sigma_{\gamma,\text{deact}}$.

Algorithm 7: UPDATETGAMSTATE($T_\gamma, \mathcal{G}, t$)

Input: T_γ is T_γ cell, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which T_γ resides
if $t = T_\gamma[t_{\text{death}}]$ **then**
  $T_\gamma[\text{dead}] \leftarrow$ **true**
else if $c[\text{TNF}] > \tau_{\text{TNF,apopt}}$ **and** with probability $p_{\text{TNF,apopt}}$ **then**
  $T_\gamma[\text{dead}] \leftarrow$ **true**
else
  if $T_\gamma[\text{deact}]$ **and** $T_\gamma[t_{\text{deact}}] + \sigma_{\gamma,\text{deact}} = t$ **then**
    $T_\gamma[\text{deact}] \leftarrow$ **false**
  if $T_\gamma[\text{deact}] =$ **false**
  and c has an infected or chronically infected phagocyte
  and with probability $p_{\text{Fas,apopt}}$ **then**
    Let M be the phagocyte in c
    Kill half of the intracellular bacteria of M
    Disperse the other half over the Moore neighborhood of c
    $M[\text{dead}] \leftarrow$ **true**
    CONTRIBUTETOCASEATION(c)

2.1.4 Macrophage

Macrophages have the ability to phagocytose extracellular bacteria. In case of Mtb, the bacteria is not necessarily cleared. Therefore we keep track of the number of intracellular bacteria in a macrophage, which we denote by B_I. There are four states attainable by a macrophage: resting, infected, chronically infected and active. In addition to these four states, there are two transcription factors: NFκB and STAT1. In case a macrophage is down-regulated by a T_{reg} cell, the property 'deact' is set and the time at which the macrophage was down-regulated is denoted by t_{deact}. The time span during which a macrophage remains down-regulated is a parameter denoted by $\sigma_{\text{P,deact}}$.

Algorithm 8: UPDATEMACSTATE($M, \mathcal{G}, t$)

Input: M is a macrophage, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which M resides
if $t = M[t_{\text{death}}]$ **then**
  $c[B_E] \leftarrow c[B_E] + M[B_I]$
  if M[state] = `Active` **then**
    CONTRIBUTETOCASEATION(c)
  M[dead] $\leftarrow$ **true**
else if c[TNF] $> \tau_{\text{TNF,apopt}}$ **and** with probability $p_{\text{TNF,apopt}}$ **then**
  Kill half of the intracellular bacteria of M
  Disperse the other half over the Moore neighborhood of c
  M[dead] $\leftarrow$ **true**
else
  if M[deact] **and** $M[t_{\text{deact}}] + \sigma_{\text{P,deact}} = t$ **then**
    M[deact] $\leftarrow$ **false**
  if M[deact] = **false then**
    Set M[NFκB] to **true** if the state is infected or active, or the number of extracellular bacteria in the Moore neighborhood is larger than $N_{\text{P,Be,NF}\kappa\text{B}}$. Otherwise set M[NFκB] to **false**.
    switch M[state] **do**
      case `Resting` HANDLEMACRESTING($M, \mathcal{G}, t$)
      case `Infected` HANDLEMACINFECTED($M, \mathcal{G}, t$)
      case `Chronically infected` HANDLEMACCHRONICALLYINFECTED($M, \mathcal{G}, t$)
      case `Active` HANDLEMACACTIVE($M, \mathcal{G}, t$)

In case a macrophage dies due to aging, its intracellular bacteria are released to the micro-compartment the macrophage resides in. In addition, when the macrophage is in the active state, its death will contribute to caseation. Should a micro-compartment become caseous, i.e. the number of killings equals the parameter N_{caseous}, any T cell residing in that micro-compartment is also killed. The second cause of death is due to TNF-α-induced apoptosis (programmed cell death). This happens only when the number of TNF-α molecules in the micro-compartment is more than $\tau_{\text{TNF,apopt}}$ and on top of that a probability of $p_{\text{TNF,apopt}}$ is satisfied. In contrast to

death by aging, TNF-α-induced apoptosis kills half of the intracellular bacteria and the other half is dispersed over the Moore neighborhood. Macrophages that are not down-regulated can execute their state-specific rules. Before this is done, the NFκB property is reevaluated; it is enabled if

- the macrophage is infected or active, or
- the number of TNF-α molecules in the macrophage's micro-compartment is more than $\tau_{\mathrm{P,TNF,NF}\kappa\mathrm{B}}$, or
- the number of extracellular bacteria in the macrophage's Moore neighborhood is more than $N_{\mathrm{P,Be,NF}\kappa\mathrm{B}}$.

If none of the previous conditions are met, the NFκB property is switched off. The pseudo code corresponding to this description is given in Algorithm 8. In the next paragraphs we will look at the state-specific rules.

Algorithm 9: HANDLEMACRESTING($M, \mathcal{G}, t$)

Input: M is a resting macrophage, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which M resides
if $c[B_E] \leq 1$ **then**
  `/* Kill an extracellular bacterium if there is only 1 (or less) */`
  $c[B_E] \leftarrow 0$
  $M[\mathrm{state}'] \leftarrow$ `Resting`
else if with probability $p_{\mathrm{P,kill}}$ **then**
  `/* Kill an extracellular bacterium and remain resting */`
  $c[B_E] \leftarrow c[B_E] - 1$
  $M[\mathrm{state}'] \leftarrow$ `Resting`
else
  `/* Become infected with an extracellular bacterium */`
  $c[B_E] \leftarrow c[B_E] - 1$
  $M[B_I] \leftarrow 1$
  $M[\mathrm{state}'] \leftarrow$ `Infected`
Enable $M[\mathrm{STAT1}]$ with probability NUMBEROFACTIVETGAMINMOORE(c) $* p_{\mathrm{P,STAT1}}$
if $M[\mathrm{STAT1}]$ **and** $M[\mathrm{NF}\kappa\mathrm{B}]$ **then**
  $M[B_I] \leftarrow 0$
  `/* Activated macrophages have a shortened lifespan of 10 days */`
  $M[t_{\mathrm{death}}] \leftarrow \mathbf{min}(M[t_{\mathrm{death}}], t + 1440)$
  $M[\mathrm{state}'] \leftarrow$ `Active`

The number of extracellular bacteria is modeled as a continuous attribute of every micro-compartment. It can therefore happen that there is just a fraction of less than one bacterium present in a micro-compartment. If a resting macrophage encounters only one extracellular bacterium, or a fraction of it, the macrophage will kill the bacterium and remain resting. Otherwise, if more than one extracellular bacterium is present, two things can happen: either with prob-

ability $p_{\text{P,kill}}$ the macrophage remains resting by killing a single extracellular bacterium, or the macrophage becomes infected with that same extracellular bacterium. For a resting macrophage to become activated, both NFκB and STAT1 need to be enabled. The latter is enabled with a probability that is proportional to the number of active T_γ cells in the Moore neighborhood. In Algorithm 9 the pseudo code of this procedure is given.

Algorithm 10: HANDLEMACINFECTED($M, \mathcal{G}, t$)

Input: M is an infected macrophage, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which M resides
GROWINTMTB(M)
$p \leftarrow (N_{\text{M,Bi,CI}} - M[B_I])/100$
if $c[B_E] > 0$ **and** with probability p **then**
  `/* The less intracellular bacteria are present, the higher the probability for taking up more will be */`
  $\Delta B \leftarrow \textbf{min}(c[B_E], 1)$
  $c[B_E] \leftarrow c[B_E] - \Delta B$
  $M[B_I] \leftarrow M[B_I] + \Delta B$
if $M[B_I] \geq N_{\text{M,Bi,CI}}$ **then**
  `/* Become chronically infected, if the number of intracellular bacteria exceeds` $N_{\text{M,Bi,CI}}$ `*/`
  $M[\text{state}'] \leftarrow$ `Chronically infected`
else
  Enable M[STAT1] with probability NUMBEROFACTIVETGAMINMOORE(c) $* p_{\text{P,STAT1}}$
  if M[STAT1] **and** M[NFκB] **then**
    $M[B_I] \leftarrow 0$
    `/* Activated macrophages have a shortened lifespan of 10 days */`
    $M[t_{\text{death}}] \leftarrow \textbf{min}(M[t_{\text{death}}], t + 1440)$
    $M[\text{state}'] \leftarrow$ `Active`
  else
    $M[\text{state}'] \leftarrow$ `Infected`

We can see in Algorithm 10 that an infected macrophage is still able to phagocytose extracellular bacteria. The probability of this occurring, however, decreases with the number of intracellular bacteria present in the infected macrophage (see Figure 1). Activation of an infected macrophage occurs when both NFκB and STAT1 are enabled; the latter is enabled with a probability proportional to the number of active T_γ cells in the Moore neighborhood, whereas the former depends on TNF-α in the local micro-compartment and extracellular bacteria in the neighborhood. When the number of intracellular bacteria exceeds the parameter $N_{\text{M,Bi,CI}}$, the macrophage becomes chronically infected. At that point the fate of the macrophage is sealed: the intracellular bacteria will continue to reproduce until they grow beyond $N_{\text{M,Bi,burst}}$ causing the macrophage to burst (see Algorithm 11). The bursting contributes to caseation of the local micro-compartment and the dispersion of all intracellular bacteria over the Moore neighborhood.

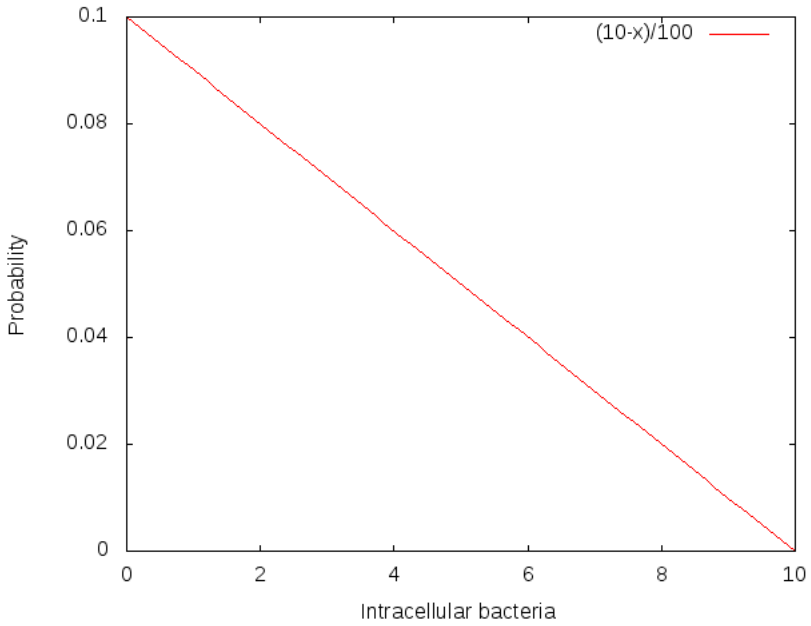


Figure 1: Probability of taking up bacteria decreases, as the number of intracellular bacteria increases; in this plot we have that $N_{\mathrm{M,Bi,CI}} = 10$.

Algorithm 11: HandleMacChronicallyInfected$(M, \mathcal{G}, t)$

Input: M is a chronically infected macrophage, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which M resides
GrowIntMtb(M)
if $M[B_I] \geq N_{\mathrm{M,Bi,burst}}$ **then**
  Disperse all intracellular bacteria over the Moore neighborhood of c
  $M[\mathrm{dead}] \leftarrow$ **true**
  ContributeToCaseation(c)
else
  $M[\mathrm{state}'] \leftarrow$ `Chronically infected`

An activated macrophage has a shortened lifespan of at most 10 days. During its remaining lifespan it has the ability to kill extracellular bacteria in its local micro-compartment. Unlike a resting macrophage, an activated macrophage can kill more than one extracellular bacterium (as many as $N_{\mathrm{P,Be,kill}}$) without being restricted by the total number of extracellular bacteria in its local micro-compartment (see Algorithm 12).

Algorithm 12: HANDLEMACACTIVE($M, \mathcal{G}, t$)

Input: M is an active macrophage, $\mathcal{G}$ is a uniform grid and t is the current time step

1 Let c denote the micro-compartment in which M resides
2 $c[B_E] \leftarrow c[B_E] - \mathbf{min}(c[B_E], N_{\mathrm{P,Be,kill}})$
3 $M[\mathrm{state}'] \leftarrow$ `Active`

2.1.5 Microglia

In our model we only consider ramified and reactivated microglia. The latter are further classified in the following four states: resting, infected, chronically infected or activated. In a reactivated microglial cell, the two transcription factors NFκB and STAT1 can be enabled. For TNF-α-induced apoptosis to occur the same pre-conditions as with macrophages have to be met: the level of TNF-α in the local micro-compartment has to be above $\tau_{\mathrm{TNF,apopt}}$ and a probability of $p_{\mathrm{TNF,apopt}}$ should be satisfied. An apoptosed microglial cell releases half of its intracellular bacteria to its Moore neighborhood; the other half is killed. The second cause of microglial death is due to aging. In the model microglia live — just like macrophages — at most 100 days. Death of an activated microglial cell contributes to caseation.

Microglia can be down-regulated by T_{reg} cells, in which case they stop performing their state-specific rules for a period of $\sigma_{\mathrm{m,deact}}$ time steps. Microglia that are not down-regulated perform their state-specific rules after updating the NFκB property. NFκB is turned on if

- the microglial cell is infected or active, or
- the number of TNF-α molecules in the micro-compartment of the microglial cell is more than $\tau_{\mathrm{m,TNF,NF}\kappa\mathrm{B}}$, or
- the number of extracellular bacteria in the Moore neighborhood of the microglial cell is more than $N_{\mathrm{m,Be,NF}\kappa\mathrm{B}}$.

If none of the above conditions are met, the NFκB property is disabled. In Algorithm 13 the pseudo code corresponding to this description is given.

Algorithm 13: UPDATEMGSTATE($m, \mathcal{G}, t$)

Input: m is a microglial cell, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which m resides
if $t = m[t_{\text{death}}]$ **then**
  $c[B_E] \leftarrow c[B_E] + m[B_I]$
  if m[state] = `Active` **then**
    CONTRIBUTETOCASEATION(c)
  m[dead] $\leftarrow$ **true**
else if c[TNF] $> \tau_{\text{TNF,apopt}}$ **and** with probability $p_{\text{TNF,apopt}}$ **then**
  Kill half of the intracellular bacteria of m
  Disperse the other half over the Moore neighborhood of c
  m[dead] $\leftarrow$ **true**
else
  if m[deact] **and** $m[t_{\text{deact}}] + \sigma_{\text{m,deact}} = t$ **then**
    m[deact] $\leftarrow$ **false**
  if m[deact] = **false then**
    Set m[NFκB] to **true** if the state is infected or active, or the number of extracellular bacteria in the Moore neighborhood is larger than $N_{\text{m,Be,NF}\kappa\text{B}}$. Otherwise set m[NFκB] to **false**.
    switch m[state] **do**
      case `Ramified` HANDLEMGRAMIFIED($m, \mathcal{G}, t$)
      case `Resting` HANDLEMGRESTING($m, \mathcal{G}, t$)
      case `Infected` HANDLEMGINFECTED($m, \mathcal{G}, t$)
      case `Chronically infected` HANDLEMGCHRONICALLYINFECTED($m, \mathcal{G}, t$)
      case `Active` HANDLEMGACTIVE($m, \mathcal{G}, t$)

A ramified microglial cell is stationary and does not actively participate in the immune response. It can become reactivated in the following two ways. If the TNF-α level is above $\tau_{\text{m,TNF,react}}$, the microglial cell in question becomes resting [9]. The other way involves extracellular bacteria in the Moore neighborhood [9]: if there is an extracellular bacterium in the Moore neighborhood, the ramified microglial cell becomes infected with that bacterium (see Algorithm 14).

Algorithm 14: HANDLEMGRAMIFIED($m, \mathcal{G}, t$)

Input: m is a ramified macrophage, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which m resides
if c[TNF] $> \tau_{\text{m,TNF,react}}$ **then**
  m[state$'$] $\leftarrow$ `Resting`
else if there are extracellular Mtb in the Moore neighborhood of c **then**
  Let c' be a micro-compartment in the Moore neighborhood of c containing at least one extracellular bacterium (i.e. $c'[B_E] \geq 1$)
  $c'[B_E] \leftarrow c'[B_E] - 1$
  $m[B_I] \leftarrow 1$
  m[state$'$] $\leftarrow$ `Infected`
else
  m[state$'$] $\leftarrow$ `Ramified`

Algorithm 15: HANDLEMGRESTING($m, \mathcal{G}, t$)

Input: m is a resting microglial cell, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which m resides
if $c[B_E] \leq 1$ **then**
    `/* Kill an extracellular bacterium if there is only 1 (or less) */`
    $c[B_E] \leftarrow 0$
    $m[\text{state}'] \leftarrow$ `Resting`
else if with probability $p_{\text{m,kill}}$ **then**
    `/* Kill an extracellular bacterium and remain resting */`
    $c[B_E] \leftarrow c[B_E] - 1$
    $m[\text{state}'] \leftarrow$ `Resting`
else
    `/* Become infected with an extracellular bacterium */`
    $c[B_E] \leftarrow c[B_E] - 1$
    $m[B_I] \leftarrow 1$
    $m[\text{state}'] \leftarrow$ `Infected`
Enable m[STAT1] with probability NUMBEROFACTIVETGAMINMOORE(c) $* p_{\text{m,STAT1}}$
if m[STAT1] **and** m[NFκB] **then**
    $m[B_I] \leftarrow 0$
    `/* Activated microglia have a shortened lifespan of 10 days */`
    $m[t_{\text{death}}] \leftarrow \mathbf{min}(m[t_{\text{death}}], t + 1440)$
    $m[\text{state}'] \leftarrow$ `Active`

A resting microglial cell has the ability to kill an extracellular bacterium. If there is only one bacterium (or a fraction of less than one) in the local micro-compartment of the microglial cell, the bacterium is killed. If there are several bacteria in the local micro-compartment then two things can happen: either with a probability of $p_{\text{m,kill}}$ one of the bacteria is killed by the microglial cell which afterwards will remain in the resting state, or the microglial cell becomes infected with one bacterium. The transcription factor STAT1 is enabled with a probability that is proportional to the number of non-down-regulated T_γ cells in the Moore neighborhood. In case both transcription factors — NFκB and STAT1— are enabled, the microglial cells becomes activated (see Algorithm 15).

We can see in Algorithm 16 that the rules concerning infected microglia are the same as those of infected macrophages. Infected microglia have the ability to uptake more extracellular bacteria with a probability that is inversely proportional to the number of intracellular bacteria (see Figure 1). Should the number of intracellular bacteria exceed $N_{\text{m,Bi,CI}}$, the microglial cell in question becomes chronically infected. If this is not the case, there is still a chance for the microglial cell to become activated. This happens when both transcription factors NFκB and STAT1 are enabled. The latter is enabled in the same way as in resting microglia and macrophages.

In case a microglial cell is chronically infected, its intracellular bacteria will continue to re-

Algorithm 16: HANDLEMGINFECTED($m, \mathcal{G}, t$)

Input: m is an infected microglial cell, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which m resides
GROWINTMTB(m)
$p \leftarrow (N_{\mathrm{m,Bi,CI}} - m[B_I])/100$
if $c[B_E] > 0$ **and** with probability p **then**
  `/* The less intracellular bacteria are present, the higher the probability for taking up more will be */`
  $\Delta B \leftarrow \mathbf{min}(c[B_E], 1)$
  $c[B_E] \leftarrow c[B_E] - \Delta B$
  $m[B_I] \leftarrow m[B_I] + \Delta B$
if $m[B_I] \geq N_{\mathrm{m,Bi,CI}}$ **then**
  `/* Become chronically infected, if the number of intracellular bacteria exceeds` $N_{\mathrm{m,Bi,CI}}$ `*/`
  $m[\mathrm{state}'] \leftarrow$ `Chronically infected`
else
  Enable m[STAT1] with probability NUMBEROFACTIVETGAMINMOORE(c) $* p_{\mathrm{m,STAT1}}$
  if m[STAT1] **and** m[NFκB] **then**
    $m[B_I] \leftarrow 0$
    `/* Activated microglia have a shortened lifespan of 10 days */`
    $m[t_{\mathrm{death}}] \leftarrow \mathbf{min}(m[t_{\mathrm{death}}], t + 1440)$
    $m[\mathrm{state}'] \leftarrow$ `Active`
  else
    $m[\mathrm{state}'] \leftarrow$ `Infected`

Algorithm 17: HANDLEMGCHRONICALLYINFECTED($m, \mathcal{G}, t$)

Input: m is a chronically infected microglial cell, $\mathcal{G}$ is a uniform grid and t is the current time step

Let c denote the micro-compartment in which m resides
GROWINTMTB(m)
if $m[B_I] \geq N_{\mathrm{m,Bi,burst}}$ **then**
  Disperse all intracellular bacteria over the Moore neighborhood of c
  m[dead] $\leftarrow$ **true**
  CONTRIBUTETOCASEATION(c)
else
  $m[\mathrm{state}'] \leftarrow$ `Chronically infected`

produce until they grow beyond $N_{\mathrm{m,Bi,burst}}$, at which point the microglial cell bursts and all intracellular bacteria are dispersed over the Moore neighborhood. The bursting of a chronically infected microglial cell contributes to caseation. In Algorithm 17 the pseudo code corresponding to this procedure is given.

Algorithm 18: HANDLEMGACTIVE($m, \mathcal{G}, t$)

Input: m is an active microglial cell, $\mathcal{G}$ is a uniform grid and t is the current time step

1 Let c denote the micro-compartment in which m resides
2 $c[B_E] \leftarrow c[B_E] - \mathbf{min}(c[B_E], N_{\mathrm{m,Be,kill}})$
3 $m[\mathrm{state}'] \leftarrow$ `Active`

Activated microglial cells excel at killing extracellular bacteria: every time step $N_{\mathrm{m,Be,kill}}$ extracellular bacteria are killed in the local micro-compartment. The price that microglia pay for this increased phagocytic ability is that they have a shortened life span of at most 10 days (see line 15 of Algorithm 15 and line 16 of Algorithm 16). The pseudo code corresponding to the state transition of activated microglia is given in Algorithm 18.

2.2 Cytokine/chemokine specific rules

In this subsection we look at the rules concerning the chemokines CCL2, CCL5, CXCL9/10/11 and the cytokine TNF-α. We start by describing how they are secreted, diffused and degraded, followed by a description of their effects on recruitment, proliferation and movement of immune cells.

2.2.1 Secretion

In addition to macrophages, also microglia have the ability to secrete the chemokines CCL2, CCL5, CXCL9/10/11 and the cytokine TNF-α [7, 10, 11]. For both types of phagocytes, secretion is dependent on the transcription factor NFκB and the state of the phagocyte. For instance, ramified microglia do not secrete at all, whereas resting microglia that have NFκB enabled secrete at half rate. In all other cases where NFκB is enabled, secretion happens at maximal rate. Infected microglia secrete at half rate if NFκB is disabled. The rates by which secretion occurs for TNF-α, CCL2, CCL5 and CXCL9 are denoted by $s_{\mathrm{P,TNF}}$, $s_{\mathrm{P,CCL2}}$, $s_{\mathrm{P,CCL5}}$ and $s_{\mathrm{P,CXCL9}}$, respectively. The secretion concerning macrophages are the same as for microglia. In Algorithms 19 and 20 the pseudo code corresponding to secretion by microglia is given. T_γ and T_{cyt} that are not down-regulated secrete TNF-α. In Algorithm 21 details are given.

Algorithm 19: MacSecrete($\mathcal{G}$)

Input: $\mathcal{G}$ is a uniform grid

for $i \leftarrow 1$ **to** 100 **do**
  for $j \leftarrow 1$ **to** 100 **do**
    if $\mathcal{G}_{ij}$ has a macrophage M **and not** M[deact] **then**
      Let c be the micro-compartment at $\mathcal{G}_{ij}$
      if M[NFκB] **and** M[state] $\neq$ `Resting` **then**
        c[TNF] $\leftarrow c$[TNF] $+ s_{\mathrm{P,TNF}}$
        c[CCL2] $\leftarrow c$[CCL2] $+ s_{\mathrm{P,CCL2}}$
        c[CCL5] $\leftarrow c$[CCL5] $+ s_{\mathrm{P,CCL5}}$
        c[CXCL9] $\leftarrow c$[CXCL9] $+ s_{\mathrm{P,CXCL9}}$
      else if (M[NFκB] **and** M[state] = `Resting`) **or** M[state] = `Infected` **then**
        c[TNF] $\leftarrow c$[TNF] $+ 0.5 \cdot s_{\mathrm{P,TNF}}$
        c[CCL2] $\leftarrow c$[CCL2] $+ 0.5 \cdot s_{\mathrm{P,CCL2}}$
        c[CCL5] $\leftarrow c$[CCL5] $+ 0.5 \cdot s_{\mathrm{P,CCL5}}$
        c[CXCL9] $\leftarrow c$[CXCL9] $+ 0.5 \cdot s_{\mathrm{P,CXCL9}}$

Algorithm 20: MgSecrete($\mathcal{G}$)

Input: $\mathcal{G}$ is a uniform grid

for $i \leftarrow 1$ **to** 100 **do**
  for $j \leftarrow 1$ **to** 100 **do**
    if $\mathcal{G}_{ij}$ has a microglial cell m **and not** m[deact] **then**
      Let c be the micro-compartment at $\mathcal{G}_{ij}$
      if m[state] $\neq$ `Ramified` **then**
        if m[NFκB] **and** m[state] $\neq$ `Resting` **then**
          c[TNF] $\leftarrow c$[TNF] $+ s_{\mathrm{P,TNF}}$
          c[CCL2] $\leftarrow c$[CCL2] $+ s_{\mathrm{P,CCL2}}$
          c[CCL5] $\leftarrow c$[CCL5] $+ s_{\mathrm{P,CCL5}}$
          c[CXCL9] $\leftarrow c$[CXCL9] $+ s_{\mathrm{P,CXCL9}}$
        else if (m[NFκB] **and** m[state] = `Resting`) **or** m[state] = `Infected` **then**
          c[TNF] $\leftarrow c$[TNF] $+ 0.5 \cdot s_{\mathrm{P,TNF}}$
          c[CCL2] $\leftarrow c$[CCL2] $+ 0.5 \cdot s_{\mathrm{P,CCL2}}$
          c[CCL5] $\leftarrow c$[CCL5] $+ 0.5 \cdot s_{\mathrm{P,CCL5}}$
          c[CXCL9] $\leftarrow c$[CXCL9] $+ 0.5 \cdot s_{\mathrm{P,CXCL9}}$

Algorithm 21: TcellSecrete($\mathcal{G}$)

Input: $\mathcal{G}$ is a uniform grid

for $i \leftarrow 1$ **to** 100 **do**
  for $j \leftarrow 1$ **to** 100 **do**
    if $\mathcal{G}_{ij}$ has a $T_\gamma/T_{\mathrm{reg}}$ cell t **and not** t[deact] **then**
      Let c be the micro-compartment at $\mathcal{G}_{ij}$
      if t is a T_γ cell **then**
        c[TNF] $\leftarrow c$[TNF] $+ 0.5 \cdot s_{\gamma,\mathrm{TNF}}$
      if t is a T_{cyt} cell **then**
        c[TNF] $\leftarrow c$[TNF] $+ 0.5 \cdot s_{\mathrm{c,TNF}}$

2.2.2 Diffusion

In every 6-second time step, secretion is followed by diffusion. We perform diffusion using a forward Euler scheme. The pseudo code is given in Algorithm 22.

Algorithm 22: DIFFUSE($\mathcal{G}$)

Input: $\mathcal{G}$ is a uniform grid; the micro-compartment at the bottom-left corner is $\mathcal{G}_{1,1}$

Let $\mathcal{G}'$ be a uniform grid of the same dimensions as $\mathcal{G}$
$\mu_{\text{TNF}} \leftarrow \frac{6 \cdot D_{\text{TNF}}}{4 \cdot 10^{-6}}$
$\mu_{\text{CC}} \leftarrow \frac{6 \cdot D_{\text{CC}}}{4 \cdot 10^{-6}}$
$c_0[\text{TNF}] \leftarrow 0$
$c_0[\text{CCL2}] \leftarrow 0$
$c_0[\text{CCL5}] \leftarrow 0$
$c_0[\text{CXCL9}] \leftarrow 0$
for $i \leftarrow 1$ **to** 100 **do**
  for $j \leftarrow 1$ **to** 100 **do**
    Let c and c' be the micro-compartments at $\mathcal{G}_{ij}$ and $\mathcal{G}'_{ij}$, respectively
    if $i = 1$ **then** $c_{\text{down}} \leftarrow c_0$ **else** $c_{\text{down}} \leftarrow \mathcal{G}_{i-1,j}$
    if $i = 100$ **then** $c_{\text{up}} \leftarrow c_0$ **else** $c_{\text{up}} \leftarrow \mathcal{G}_{i+1,j}$
    if $j = 1$ **then** $c_{\text{left}} \leftarrow c_0$ **else** $c_{\text{left}} \leftarrow \mathcal{G}_{i,j-1}$
    if $j = 100$ **then** $c_{\text{right}} \leftarrow c_0$ **else** $c_{\text{right}} \leftarrow \mathcal{G}_{i,j+1}$
    $c'[\text{TNF}] \leftarrow c[\text{TNF}] + \mu_{\text{TNF}} \cdot (c_{\text{down}}[\text{TNF}] + c_{\text{up}}[\text{TNF}] + c_{\text{left}}[\text{TNF}] + c_{\text{right}}[\text{TNF}])$
    $c'[\text{CCL2}] \leftarrow c[\text{CCL2}] + \mu_{\text{CC}} \cdot (c_{\text{down}}[\text{CCL2}] + c_{\text{up}}[\text{CCL2}] + c_{\text{left}}[\text{CCL2}] + c_{\text{right}}[\text{CCL2}])$
    $c'[\text{CCL5}] \leftarrow c[\text{CCL5}] + \mu_{\text{CC}} \cdot (c_{\text{down}}[\text{CCL5}] + c_{\text{up}}[\text{CCL5}] + c_{\text{left}}[\text{CCL5}] + c_{\text{right}}[\text{CCL5}])$
    $c'[\text{CXCL9}] \leftarrow c[\text{CXCL9}] + \mu_{\text{CC}} \cdot (c_{\text{down}}[\text{CXCL9}] + c_{\text{up}}[\text{CXCL9}] + c_{\text{left}}[\text{CXCL9}] + c_{\text{right}}[\text{CXCL9}])$
$\mathcal{G} \leftarrow \mathcal{G}'$

2.2.3 Degradation

Degradation of TNF-α and the chemokines also proceeds at the finer timescale of 6-second time steps. The two parameters δ_{TNF} and δ_{CC} denote the degradation coefficients for TNF-α and the chemokines, respectively. In Algorithm 23 the pseudo code concerning degradation is given.

Algorithm 23: DEGRADE($\mathcal{G}$)

Input: $\mathcal{G}$ is a uniform grid of 100×100 micro-compartments

for $i \leftarrow 1$ **to** 100 **do**
  for $j \leftarrow 1$ **to** 100 **do**
    Let c be the micro-compartment at $\mathcal{G}_{ij}$
    $c[\text{TNF}] \leftarrow c[\text{TNF}] - \delta_{\text{TNF}} \cdot c[\text{TNF}]$
    $c[\text{CCL2}] \leftarrow c[\text{CCL2}] - \delta_{\text{CC}} \cdot c[\text{CCL2}]$
    $c[\text{CCL5}] \leftarrow c[\text{CCL5}] - \delta_{\text{CC}} \cdot c[\text{CCL5}]$
    $c[\text{CXCL9}] \leftarrow c[\text{CXCL9}] - \delta_{\text{CC}} \cdot c[\text{CXCL9}]$

2.2.4 Recruitment and proliferation

As opposed to macrophages and T cells, microglia are not recruited through vascular sources. Rather they proliferate on site. We make a distinction between ramified and reactivated microglia, as the first proliferate at a slower rate than the latter [12]. The parameter $N_{\mathrm{m,ram}}$ denotes the number of ramified microglia on the grid. This number is maintained by proliferating ramified microglia with the following probability:

$$p_{\mathrm{ram}} = \mathbf{min}\left(\frac{N_{\mathrm{m,ram}} - n_{\mathrm{ram}}}{N_{\mathrm{m,ram}}}, 0\right). \tag{1}$$

In the previous subsection we mentioned that reactive microglia can either be resting, infected, chronically infected or active. As infected and chronically infected microglia contain intracellular bacteria, we chose to allow only activated and resting microglia to proliferate. Before a resting or active microglial cell can proliferate, the TNF-α level in the local micro-compartment should be greater than $\tau_{\mathrm{m,TNF,prol}}$. To ensure that only a limited number of reactive microglia proliferate, the following probability is taken into account:

$$p_{\mathrm{react}} = \frac{N_{\mathrm{m,react,prol}}}{n_{\mathrm{res}} + n_{\mathrm{act}}}. \tag{2}$$

So $N_{\mathrm{m,react,prol}}$ is roughly the number of resting and active microglia that can proliferate each time step. In Algorithm 24 the pseudo code for the proliferation of microglia is given. We can see in Algorithm 25 that proliferation of a microglial cell results in the creation of a new microglial cell in the Moore neighborhood. In case the Moore neighborhood cannot accommodate a new microglial cell, the microglial cell in question does not proliferate. Since not much is known about the lifespan of microglia, we assign new microglial cells the same lifespan as macrophages (which is 100 days). In newly created microglia, the two transcription factors NFκB and STAT1 are turned off initially.

Macrophages and T cells are recruited through non-caseous vascular sources (see Algorithm 26). The parameter N_{M} is a lower bound on the number of *meningeal* macrophages on the grid. This lower bound is enforced during recruitment: when the number of meningeal macrophages drops below the bound, macrophages are recruited through vascular sources on the meninges. In the normal situation, i.e. when the number of macrophages is above the lower bound, macrophage

Algorithm 24: ProliferateMicroglia($\mathcal{G}, t$)

Let $\mathcal{L}_{\mathrm{m}}$ be a list containing all microglia on $\mathcal{G}$ Let n_{ram}, n_{res} and n_{act} be the number of ramified, resting and active microglia on $\mathcal{G}$, respectively
$p_{\mathrm{ram}} \leftarrow \mathbf{min}(\frac{N_{\mathrm{m,ram}} - n_{\mathrm{ram}}}{N_{\mathrm{m,ram}}}, 0)$
$p_{\mathrm{react}} \leftarrow \frac{N_{\mathrm{m,react,prol}}}{n_{\mathrm{res}} + n_{\mathrm{act}}}$
foreach $m \in \mathcal{L}_{\mathrm{m}}$ **do**
  Let c be the micro-compartment on which m resides
  if m[state] = `Ramified` **and** with probability p_{ram} **then**
    ProliferateMicroglialCell($m, \mathcal{G}, t$)
  if (m[state] = `Resting` **or** m[state] = `Active`)
  and c[TNF] $> \tau_{\mathrm{m,TNF,prol}}$ **and** with probability p_{ram} **then**
    ProliferateMicroglialCell($m, \mathcal{G}, t$)

Algorithm 25: ProliferateMicroglialCell($m, \mathcal{G}, t$)

Input: $\mathcal{G}$ is a uniform grid, m is a microglial cell and t is the current time step
Let c be a micro-compartment in the Moore neighborhood of m that can accommodate a microglial cell
if c exists **then**
  Let m' be a new microglial cell
  $m'[t_{\mathrm{birth}}] \leftarrow t$
  $m'[t_{\mathrm{death}}] \leftarrow t + 14400$
  m'[NFκB] $\leftarrow$ **false**
  m'[STAT1] $\leftarrow$ **false**
  m'[deact] $\leftarrow$ **false**
  $m'[B_I] \leftarrow 0$
  m'[state] $\leftarrow m$[state]

recruitment at a source c depends on TNF-α, CCL2 and CCL5 in the following way:

$$\alpha_{\text{TNF}} \cdot \ c[\text{TNF}] + \alpha_{\text{CCL2}} \cdot c[\text{CCL2}] + \alpha_{\text{CCL5}} \cdot c[\text{CCL5}] \geq \tau_{\text{M,recr}} \tag{3}$$

where α_{TNF}, α_{CCL2} and α_{CCL5} are configurable parameters. So the source c can recruit a macrophage when the value of the linear combination is above the threshold parameter $\tau_{\text{M,recr}}$. In addition, there is also a probability of $p_{\text{M,recr}}$ that should be satisfied. If both conditions are met, a new macrophage is recruited at c. Recall that a macrophage lives for at most 100 days [6]. We model this by assuming that all macrophages live 100 days, and we introduce some variation by setting the birth time of a macrophage, uniformly at random, to a value in the interval $[t-14400, t]$ where t is the current time step (note that 100 days corresponds to 14,400 time steps). The pseudo code corresponding to this procedure is given in Algorithm 27.

Recruitment of T cells is enabled after t_{recr} time steps and takes place at a non-caseated source c with a probability of $p_{\text{T,recr}}$, if on that source c at most one immune cell is present and one of the following conditions is satisfied: for T_γ cells

$$\alpha_{\text{TNF}} \cdot \ c[\text{TNF}] + \alpha_{\text{CCL2}} \cdot c[\text{CCL2}] + \alpha_{\text{CCL5}} \cdot c[\text{CCL5}] + \alpha_{\text{CXCL9}} \cdot c[\text{CXCL9}] \geq \tau_{\text{T,recr}}; \tag{4}$$

for T_{cyt} cells

$$\alpha_{\text{TNF}} \cdot \ c[\text{TNF}] + \alpha_{\text{CCL5}} \cdot c[\text{CCL5}] + \alpha_{\text{CXCL9}} \cdot c[\text{CXCL9}] \geq \tau_{\text{T,recr}}; \tag{5}$$

and for T_{reg} cells

$$\alpha_{\text{TNF}} \cdot \ c[\text{TNF}] + \alpha_{\text{CCL5}} \cdot c[\text{CCL5}] \geq \tau_{\text{T,recr}}. \tag{6}$$

In addition there are also probabilities associated with the recruitment of T cells: we have $p_{\text{c,recr}}$, $p_{\gamma,\text{recr}}$ and $p_{\text{reg,recr}}$ for T_{cyt}, T_γ and T_{reg} cells, respectively. We require that $p_{\text{c,recr}} + p_{\gamma,\text{recr}} + p_{\text{reg,recr}} = 1$. The maximal lifespan of a T cell is 3 days [8], which corresponds to 432 time steps. Similarly to macrophages, this is enforced by setting the birth time to at most 3 days in the past. The pseudo code of RecruitTcell is given in Algorithm 28. In summary, the cytokines and chemokines that play a role in the recruitment of immune cells are given in Table 3b.

	CCL2	CCL5	CXCL9/10/11
Mac	x	x	
Mg	x	x	
T_γ	x	x	x
T_{cyt}		x	x
T_{reg}		x	

(a) Chemokines that play a role in the movement of the immune cells.

	TNF	CCL2	CCL5	CXCL9/10/11
Mac	x	x	x	
T_γ	x	x	x	x
T_{cyt}	x	x	x	x
T_{reg}	x		x	

(b) Cytokines/chemokines that play a role in the recruitment of the immune cells.

Table 3: Role of TNF-α and chemokines in chemotaxis and recruitment of immune cells.

Algorithm 26: RECRUIT($\mathcal{G}, t$)

Input: $\mathcal{G}$ is a uniform grid and t is the current time step

Let $\mathcal{L}_{\text{sources}}$ be the list of vascular sources on $\mathcal{G}$ (by definition $|\mathcal{L}_{\text{sources}}| = 800$)
foreach $c \in \mathcal{L}_{\text{sources}}$ **do**
  if c[caseous] = **false then**
    if #cells at c is less than 2 **and** no macrophage or a microglial cell is present on c **then**
      RECRUITMAC($c, \mathcal{G}, t$)
    if #cells at c is less than 2 **and** $t \geq t_{\text{recr}}$ **then**
      RECRUITTCELL($c, \mathcal{G}, t$)

Algorithm 27: RECRUITMAC($c, \mathcal{G}, t$)

Input: c is a non-caseated source not containing a macrophage or a microglial cell and t is the current time step

Let n_{macs} be the number of macrophages on the meninges of $\mathcal{G}$
$b \leftarrow \alpha_{\text{TNF}} \cdot\ c[\text{TNF}] + \alpha_{\text{CCL2}} \cdot c[\text{CCL2}] + \alpha_{\text{CCL5}} \cdot c[\text{CCL5}] \geq \tau_{\text{M,recr}}$
if (b **and** with probability $p_{\text{M,recr}}$) **or** ($n_{\text{macs}} < N_{\text{M}}$ **and** c[meninges]) **then**
  Let M be a new macrophage on micro-compartment c
  Set $M[t_{\text{birth}}]$ uniformly at random to a value in $[t - 14400, t]$
  $M[t_{\text{death}}] \leftarrow M[t_{\text{birth}}] + 14400$
  $M[\text{state}] \leftarrow$ `Resting`
  $M[\text{deact}] \leftarrow$ **false**
  $M[\text{NF}\kappa\text{B}] \leftarrow$ **false**
  $M[\text{STAT1}] \leftarrow$ **false**
  $M[B_I] \leftarrow 0$

Algorithm 28: RecruitTCell($c, \mathcal{G}, t$)

Input: c is a non-caseated source not containing a macrophage and t is the current time step

$b_\gamma \leftarrow \alpha_{\text{TNF}} \cdot\ c[\text{TNF}] + \alpha_{\text{CCL2}} \cdot c[\text{CCL2}] + \alpha_{\text{CCL5}} \cdot c[\text{CCL5}] + \alpha_{\text{CXCL9}} \cdot c[\text{CXCL9}] \geq \tau_{\text{T,recr}}$
$b_{\text{c}} \leftarrow \alpha_{\text{TNF}} \cdot\ c[\text{TNF}] + \alpha_{\text{CCL5}} \cdot c[\text{CCL5}] + \alpha_{\text{CXCL9}} \cdot c[\text{CXCL9}] \geq \tau_{\text{T,recr}}$
$b_{\text{reg}} \leftarrow \alpha_{\text{TNF}} \cdot\ c[\text{TNF}] + \alpha_{\text{CCL5}} \cdot c[\text{CCL5}] \geq \tau_{\text{T,recr}}$
if with probability $p_{\text{T,recr}}$ **then**
  Let p be a random real number in the range $[0, 1]$
  if b_γ **and** $p \in [0, p_{\gamma,\text{recr}}]$ **then**
    Let T_γ be a T_γ cell on micro-compartment c
    Set $T_\gamma[t_{\text{birth}}]$ uniformly at random to a value in $[t - 432, t]$
    $T_\gamma[t_{\text{death}}] \leftarrow T_\gamma[t_{\text{birth}}] + 432$
    $T_\gamma[\text{deact}] \leftarrow$ **false**
  else if b_{c} **and** $p \in [p_{\gamma,\text{recr}}, p_{\gamma,\text{recr}} + p_{\text{c,recr}}]$ **then**
    Let T_{cyt} be a T_{cyt} cell on micro-compartment c
    Set $T_{\text{cyt}}[t_{\text{birth}}]$ uniformly at random to a value in $[t - 432, t]$
    $T_{\text{cyt}}[t_{\text{death}}] \leftarrow T_{\text{cyt}}[t_{\text{birth}}] + 432$
    $T_{\text{cyt}}[\text{deact}] \leftarrow$ **false**
  else if b_{reg} **and** $p \in [p_{\gamma,\text{recr}} + p_{\text{c,recr}}, p_{\gamma,\text{recr}} + p_{\text{c,recr}} + p_{\text{reg,recr}}]$ **then**
    Let T_{reg} be a T_{reg} cell on micro-compartment c
    Set $T_{\text{reg}}[t_{\text{birth}}]$ uniformly at random to a value in $[t - 432, t]$

2.2.5 Movement

The speed by which phagocytes move depends on their state. For instance, chronically infected phagocytes and ramified microglia do not move at all. Whereas resting, infected and active phagocytes move every $v_{\text{P,r}}$, $v_{\text{P,i}}$, $v_{\text{P,a}}$ time steps, respectively (see Algorithm 29 and 30). In Algorithm 31, we see that T cells move every time step.

Algorithm 29: MoveMacrophages($\mathcal{G}, t$)

Input: $\mathcal{G}$ is a uniform grid and t is the current time step

Let $\mathcal{L}_{\text{M}}$ be a list containing all the macrophages
foreach $M \in \mathcal{L}_{\text{M}}$ **do**
  if $M[\text{state}] =$ `Resting` **and** t **mod** $v_{\text{P,r}} = 0$ **then**
    MoveImmuneCell($M, \mathcal{G},$ **true**, **false**, **true**, 1.5)
  if $M[\text{state}] =$ `Active` **and** t **mod** $v_{\text{P,a}} = 0$ **then**
    MoveImmuneCell($M, \mathcal{G},$ **true**, **false**, **true**, 1.5)
  if $M[\text{state}] =$ `Infected` **and** t **mod** $v_{\text{P,i}} = 0$ **then**
    MoveImmuneCell($M, \mathcal{G},$ **true**, **false**, **true**, 1.5)

The Moore neighborhood of every micro-compartment consists of nine compartments. An immune cell has the ability to move to every micro-compartment of its Moore neighborhood, provided that micro-compartment is not caseated nor occupied. Due to chemotaxis, there is a bias toward moving to micro-compartments with higher levels of chemokines. In Table 3a, the chemokines that influence the movement of the various cell types are shown.

Whenever an immune cells wants to move, we sum for every micro-compartment in its Moore

Algorithm 30: MoveMicroglia($\mathcal{G}, t$)

Input: $\mathcal{G}$ is a uniform grid and t is the current time step

Let $\mathcal{L}_{\mathrm{m}}$ be a list containing all microglia on $\mathcal{G}$
foreach $m \in \mathcal{L}_{\mathrm{m}}$ **do**
  if m[state] = `Resting` **and** t **mod** $v_{\mathrm{P,r}} = 0$ **then**
    MoveImmuneCell($m, \mathcal{G}$, **true**, **false**, **true**, 1.5)
  if M[state] = `Active` **and** t **mod** $v_{\mathrm{P,a}} = 0$ **then**
    MoveImmuneCell($m, \mathcal{G}$, **true**, **false**, **true**, 1.5)
  if M[state] = `Infected` **and** t **mod** $v_{\mathrm{P,i}} = 0$ **then**
    MoveImmuneCell($m, \mathcal{G}$, **true**, **false**, **true**, 1.5)

Algorithm 31: MoveTcells($\mathcal{G}, t$)

Input: $\mathcal{G}$ is a uniform grid and t is the current time step

Let $\mathcal{L}_{\gamma}$, $\mathcal{L}_{\mathrm{c}}$ and $\mathcal{L}_{\mathrm{reg}}$ be lists containing all T_{γ} cells, T_{cyt} cells and T_{reg} cells, respectively
foreach $T_{\gamma} \in \mathcal{L}_{\gamma}$ **do**
  MoveImmuneCell($T_{\gamma}, \mathcal{G}$, **true**, **true**, **true**, 1)
foreach $T_{\mathrm{cyt}} \in \mathcal{L}_{\mathrm{c}}$ **do**
  MoveImmuneCell($T_{\mathrm{cyt}}, \mathcal{G}$, **false**, **true**, **true**, 1)
foreach $T_{\mathrm{reg}} \in \mathcal{L}_{\mathrm{reg}}$ **do**
  MoveImmuneCell($T_{\mathrm{reg}}, \mathcal{G}$, **false**, **true**, **false**, 1)

neighborhood the levels of the involved chemokines. In case the immune cell is a phagocyte, we multiply the highest chemokine level by 1.5 — in other words, there is an even stronger bias for phagocytes to move to micro-compartments with the highest chemokine levels. Subsequently, the sums are normalized so that each micro-compartment has a probability of moving onto it. Using the probabilities a micro-compartment c is picked. If that micro-compartment is caseous or cannot accommodate the immune cell, the immune cell does not move. Otherwise, in case the immune cell is a phagocyte it moves to c. In case the immune cell is a T cell and c is not empty, then there are additional probabilities to be met: if c contains a phagocyte, the probability of moving to c is $p_{\mathrm{T,P}}$; should c contain a T cell then the probability of moving to it is $p_{\mathrm{T,T}}$.

There are several ways to deal with movement in a non-toroidal grid. One way would be to prevent cells from moving off the grid. This, however, would lead to crowding near the edges and corners of the grid. To deal with this issue, we chose to stop tracking immune cells as soon as they leave the grid. In order to model meningeal macrophages, we enforce that a macrophage residing on a micro-compartment that is part of the meninges can only move to other meningeal micro-compartments or off the grid. The pseudo code corresponding to the movement of an immune cell is given in Algorithm 32.

Algorithm 32: MoveImmuneCell($ic, \mathcal{G}, b_{\text{CCL2}}, b_{\text{CCL5}}, b_{\text{CXCL9}}, \alpha$)

Input: ic is an immune cell on the uniform grid $\mathcal{G}$; b_{CCL2}, b_{CCL5}, b_{CXCL9} are flags denoting the involved chemokines and α is a bonus coefficient

Let $\mathcal{G}_{kl}$ be the micro-compartment on which ic resides
for $i \leftarrow -1$ **to** 1 **do**
  for $j \leftarrow -1$ **to** 1 **do**
    $p_{ij} \leftarrow \epsilon$
    if b_{CCL2} **then** $p_{ij} \leftarrow p_{ij} + \mathcal{G}_{k+i,l+j}$[CCL2]
    if b_{CCL5} **then** $p_{ij} \leftarrow p_{ij} + \mathcal{G}_{k+i,l+j}$[CCL5]
    if b_{CXCL9} **then** $p_{ij} \leftarrow p_{ij} + \mathcal{G}_{k+i,l+j}$[CXCL9]
Multiply the highest value in p by α
Normalize p
Let c be the micro-compartment picked according to the probabilities in p
if c is not on $\mathcal{G}$ **then**
  Kill ic
else if c is not caseous and has room for ic **then**
  if ic is a T cell **and** c contains a phagocyte **then**
    With probability $p_{\text{T,P}}$, move ic onto c
  else if ic is a T cell **and** c contains a T cell **then**
    With probability $p_{\text{T,T}}$, move ic onto c
  else if ic is a macrophage **and** $\mathcal{G}_{kl}$[meninges] **then**
    Only move ic onto c if c[meninges]
  else
    Move ic onto c

2.3 Mtb specific rules

Mtb prefer the intracellular environment of macrophage over the extracellular environment. It is for this reason that we make use of two different growth functions. The growth function used for intracellular bacteria is:

$$B_I(t + \Delta t) = B_I(t) + \alpha_{\text{Bi}} * B_I(t) \tag{7}$$

where α_{Bi} is a parameter denoting the growth rate of intracellular Mtb (see Figure 2a and Algorithm 33).

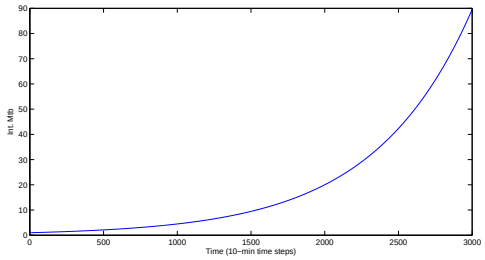
Algorithm 33: GrowIntMtb(M)

Input: M is a macrophage (or a microglial cell)

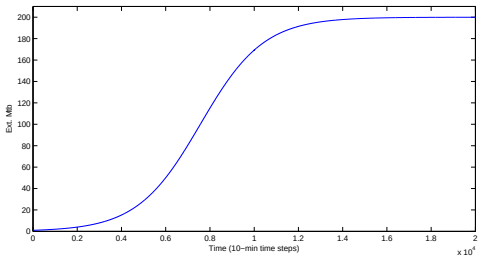
$M[B_I] \leftarrow M[B_I] + \alpha_{\text{Bi}} \cdot M[B_I]$

Growth of extracellular Mtb is bounded by the dimensions of a micro-compartment. Assuming that a micro-compartment can sustain at most K_{Be} bacteria, the following logistic growth function can be used to model growth of extracellular Mtb:

$$B_E(t + \Delta t) = B_E(t) + \alpha_{\text{Be}} \cdot B_E(t) \cdot \left(1 - \frac{B_E(t)}{K_{\text{Be}}}\right) \tag{8}$$



(a) Growth of intracellular bacteria with $B_I(0) = 1$ and $\alpha_{\text{Bi}} = 0.0015$.



(b) Growth of extracellular bacteria with $B_E(0) = 1$, $\alpha_{\text{Be}} = 0.0007$ and $K_{\text{Be}} = 200$.

Figure 2: Growth of intracellular and extracellular bacteria

where α_{Be} is a parameter denoting the growth rate of extracellular Mtb (see Figure 2b and Algorithm 34).

Algorithm 34: GROWEXTMTB($\mathcal{G}$)

Input: $\mathcal{G}$ is a uniform grid of 100×100 micro-compartments

for $i \leftarrow 1$ **to** 100 **do**
  for $j \leftarrow 1$ **to** 100 **do**
    Let c be the micro-compartment at $\mathcal{G}_{ij}$
    $c[B_E] \leftarrow c[B_E] + \alpha_{\text{Be}} \cdot c[B_E] \cdot \left(1 - \frac{c[B_E]}{K_{\text{Be}}}\right)$

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