Necrotizing Enterocolitis (NEC) Agent-Based Model (ABM) Pseudocode

The code for the model is split into various parts according to agent, with each part having its own unique sub-routines. A "tick" in the model is defined as the point when all of the code is carried out once an experiment is run by continuous ticks in succession until a given outcome is achieved.

The modeling environment consists of Agents that are free to move around and manipulate variables within themselves and on "patches." Patches can be considered immovable agents that exist as a grid in the modeling space. They can carry out specialized commands that relate to their ability to store and diffuse particular variables.

Epithelial Cell Rules

The first rule that a neonatal gut epithelial cell (NGEC) carries out is its consumption of nutrients from the environment. Nutrition is generated as the output of a Hill function from a patch variable called "Replenish-rate." The Replenishment-rate variable can be controlled in real time for each experiment. This output is fed into a series of Hill functions in the following manner to achieve our model for reactive oxygen species (ROS) generation and clearance secondary to cell metabolism:

1. Hill (Replenish-rate) = Nutrition;
2. Hill (Nutrition) = Consumption;
3. Hill (Consumption) = Metabolic ROS Production.

The amount of intracellular Nitric Oxide is scaled and added to Metabolic ROS Production to achieve the amount of total stress production:

4. Hill (Metabolic ROS Production) + Nitric Oxide = Total ROS Production;
5. Hill (Total ROS Production) = ROS Clearance.

Variables within the Hill equation for ROS clearance were manipulated to determine the efficacy of ROS clearance (i.e., Stress Clearance Sensitivity and Stress Clearance Capability). To simulate the effect of ROS Clearance on Metabolic ROS Production, the outputs of both Hill equations are multiplied to achieve the final amount of ROS in the cell:

6. Total ROS Production × ROS Clearance = Total ROS.

According to the amount of total ROS left in cells, NGECs begin generating different variables for different cell signaling pathways:

7. If Total ROS < 22, then [Low Stress Function];
8. If Total ROS > 22, then [Apoptosis Function];
9. If Total ROS > 25, then [Inflammation Function].

The Low Stress Function allows maximum consumption of the available nutrients and clearance of the proteins generated by apoptosis. The Apoptosis Function triggers the generation of several variables to enact apoptosis in the following manner:

10. Increase p53 by 1;
11. If p53 > 100, then [Increase Cytochrome C by 1];
12. If Cytochrome C > 100, then [Increase Caspase by 1];
13. If Caspase > 100, then [die].

A cell that undergoes death by the Apoptosis pathway will change its shape and steadily cease metabolic function. Any given cell also has a minute chance of undergoing apoptosis spontaneously given the same mechanism.

The Inflammation Function of a cell can occur simultaneously along with the Apoptosis Function. Several variables for the generation of nuclear factor (NF)-κB and its inhibitor are manipulated in the following manner on activation of the Inflammatory pathway:

14. Decrease NF-κB-IκB by 1;
15. Increase NF-κB by 1.

Once NF-κB reaches a certain concentration, it triggers the generation of its inhibitor, IκB:

16. If NF-κB > 0, then [increase IκB mRNA by 1];
17. If IκB mRNA > 100, then [increase IκB by 1];
18. If IκB > 0, then [decrease NF-κB by 1 and increase NF-κB-Iκb by 1].

With this negative feedback, NF-κB concentrations will remain constant as long as the proper stimulus is present, while decreasing when the stimulus is absent. Once the NF-κB concentration reaches a particular point, additional variables for inflammatory products are generated:

19. If NF-κB > 100, then [increase NO· by 1 and increase TNF-α by 1].

These variables are secreted to surrounding patches, diffused, and taken up by neighboring cells. Whereas NO· diffuses freely in and out of surrounding cells, tumor necrosis factor (TNF)-α must interact with a receptor in the following manner to have its effect:

20. If TNF-α > 0, then [increase TNFα-Receptor-bound by 1];
21. If TNF-α-Receptor-bound > 0, then [Inflammatory Pathway].

In this way, inflammatory signals can cascade on each other to create a forward feedback loop. The NF-κB has an additional effect by decreasing the rate of cytochrome C generation, making it "pro-survival." The TNF-α also increases a variable for receptor-interacting protein (RIP) kinase, a signaling pathway for necrosis:

22. If TNF-αReceptor-bound > 0, then [increase RIP kinase by 1].

A cell undergoes necrosis once the RIP kinase concentration reaches its maximum and will release its NO·, TNF-α, and damage-associated molecular pattern (DAMP) variables to surrounding patches.

In addition to acting as an ROS, as previously described, NO· can exert effects on tight junction metabolism. Tight junctions are formed at baseline by cells from mRNA, translated to cytoplasmic proteins, and finally localized to cell membranes, where they exert their effects:

23. Increase Tight-Junction mRNA by 1 (at baseline);
24. If Tight-Junction mRNA is > 100, then [increase Tight-Junction Cytoplasm by 1];

25. If Tight-Junction Cytoplasm > 100, then [set shape as Cell_Tight-Junction];
26. If Tight-Junction Cytoplasm < 100, then [set shape as Cell_No-Tight Junction].

The cell shape is what bacterial agents use to determine whether they can interact with a given cell (this process is explained in detail in a later section).

An additional variable exists for Toll-like receptor (TLR)-4, which can interact with variables for DAMPs and pathogen-associated molecular patterns (PAMPs) to activate NF-κB in the same manner as TNF-α:

27. If DAMP > 0, then [increase TLR-bound by 1];
28. If PAMP > 0, then [increase TLR-bound by 1];
29. If TLR-bound > 0, then [Inflammatory Pathway].

Bacteria Rules

Bacteria are the only motile agents in the system. At baseline, they move forward by small increments with a random heading. On recognition of an NGEC having loss of tight junctions, they will adhere and begin to generate a variable representing PAMPs, which are capable of activating the cell's inflammatory pathways through TLR-4.

Goblet Cell Rules

Goblet cells contain all of the same rules as epithelial cells, as well as generating a variable for mucus. In addition to the absence of tight junctions, low mucus concentrations are required for a bacterial agent to generate pathogen-associated molecular patterns (PAMPs) capable of interacting with TLR. Mucus production by intact goblet cells proceeds at a set rate that maintains an equilibrated layer of mucus across the surface of the NEC ABM. Mucus production is affected at the system level if goblet cells undergo apoptosis or necrosis during the experiment. This leads to regions of mucus depletion, where bacteria can interact with those NGECs who have lost tight junction integrity.