ENteric Immunity SImulator: A tool for *in silico study of gut* immunopathologies

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The Goal

Whether gut infection with a certain microbe will result in immunopathology (ulcer, inflammation, bloody diarrhea, etc.) is determined by the mucosal immune response, of which there are four possibilities:

- **Complete tolerance** that leads to non-pathogenic microbe persistence
- **Hypo-inflammation** in which a pathogen is not completely eliminated
- **Inflammation** that eliminates the microbe, but ceases prior to extensive tissue damage
- **Hyper-inflammation** in which the microbe is eliminated, but at expense of host tissue destruction

Identifying mechanisms by which each response develops is key to devising treatment and intervention strategies.

Current research shows that the gut inflammatory responses cannot be readily predicted as it is influenced by microbe-mediated manipulation of immune cell function and has a complex relationship with anti-inflammatory regulatory pathways, induced constitutively by commensal bacteria and mucosal homeostatic processes.

ENISI is a tool to aide in answering key mucosal immunity-related questions:
- What is the net response to a pathogen given the complex interplay between both regulatory and inflammatory pathways?
- Which aspects of these competing pathways could be exploited to inhibit pathogen invasion, infection, and evolution?
- Which aspects of mucosal immunity allow efficient elimination of a pathogen while keeping immunopathogenic side effects to a minimum?

The Model

Populations are represented by groups of individuals with the same *cell-type* and tissue site *location.* Each cell-type (macrophage, dendritic cell, CD4+ T helper cell) that occupies a specific immunological state is represented as a population whose individuals move among tissue sties. Each cell changes its location depending on type-specific rules. When in the same location, cells are considered in contact and may change immunological state depending on a specific set of rules for interaction. Individual movement and interactions are specified as programs, the simulation is run, and high-level behaviors are observed. Experiments are conducted by modifying individual programs, to represent specific laboratory conditions, and observing the effect on the net immune response.

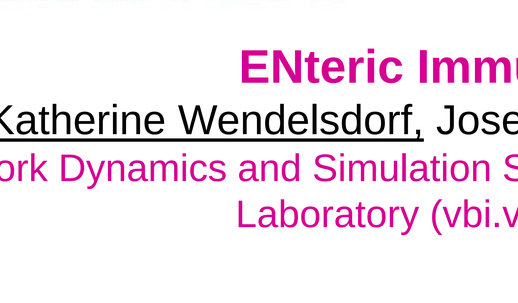


Figure 1. A State-chart description of the states that may be occupied by an individual immune cell or bacteria in our model as well as the conditions under which an individual may transition from one state to another. state-chart format. An arrow between boxes indicates a transition of that entity from one state to the other. The arrow is labeled with the event necessary for the specific transition to occur with conditions in parenthesis. An individual's 'neighbor' is any other individuals it is currently in contact with.

Overview of Implementation

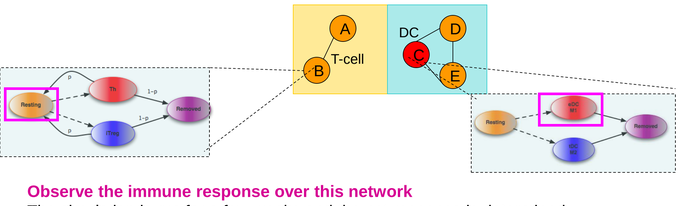
Provided a synthetic population: Each individual occupies a state defined by its specific *cell-type* (i.e. naive T-cell, commensal bacteria, etc) and l*ocation* (lumen, lamina propria, or lymphnode).

Resting CD4+ T-cell

Time	Location	contacts
30 minutes	Lymph node	cells in *l(i,j)*
30 minutes	circulation	

Derive a contact network: Cells migrate, according to a type-specific schedule. When two cells are in the same sublocation they are in contact resulting in a network composed of fully connected subgraphs in each sublocation that are updated every 6 virtual hours .

Implement state-transition function:
Immunological state of each individual cell changes according to function dependent on either i) the state of the neighbors in a cell's contact network or ii) he length of time it has occupied its current state. The state transitions may occur with a probability given these conditions are met.



Observe the immune response over this network
The simulation is run for a few weeks and the net response is determined.

Implement different experimental conditions and intervention strategies
Add pathogen at different time points, change the ability of M1 to switch to M2, change functions of cells representing specific gene expression patterns, change migration patterns, etc.

ENISI: A simulator of mucosal Inflammatory responses

We are currently developing ENISI, a tool for mucosal immunologists to test and generate hypothesized mechanisms for clinical enteric disease outcomes given in vitro observations. ENISI is an agent-based simulator of the antagonistic inflammatory and regulatory immune pathways of the gut as individual immune cells interact with and respond to commensal and foreign bacteria. Version 0.9 of ENISI will be released in the MIEP website through a user-friendly interface in September of 2011.

At this development stage ENISI represents only a subset of the relevant sites and cells of the gut mucosa. These include:

Cell-types
- Naive T-cell
- Memory T-cell: Central , Effector
- Activated T-cells: Th1 Effector (Th), Induced regulatory(iTreg)
- Natural T-regulatory cells (nTreg): Active , Resting
- Macrophages: Undifferentiated (M0), Activating (M1), Regulatory (M2)
- Dendritic Cells: Immature , Effector (eDC), Tolerogenic (tDC)
- Epithelial cells: normal , pro-inflammatory
- Foreign bacteria: *B. hyodysenteriae* (Bf)
- Tolerance-inducing Commensal bacteria (B)

Tissue Sites
Lumen
Lamina Propria (LP)

Immunological states
Resting
Active- inflammatory
Active-regulatory
Dead/Anergic

How ENISI is used
Given user-specified conditions for infection in the gut or gastric mucosa, ENISI predicts immune cells dynamics over the course of infection as well as the effect on epithelial lining. As such, output is easily compared to experimental results for the sake of testing and generating hypothesis (Figure 2). Conditions specified may include:

Infection specifics
- bacterial strains
- dose and timing of infection

Experimental host phenotypes
- susceptibility of each immune cell population to specific cytokines
- amount of various cytokines produced by different immune cell populations during infection

Host immunological set-point
- initial immune cell populations present
- microfloral demographics at the time of infection

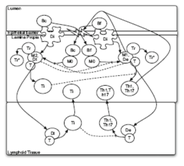
Strain-specific cell response to bacteria
- Effect of bacteria on inducing effector vs. tolerogenic response in antigen presenting cells
- Expression of various factors by epithelial cells: inflammatory cytokines, chemoattractants, defensins, etc.

Figure 2 General process for generating and testing hypothesis with ENISI

Ulcerative colitis associated with individuals with NOD2 mutant genotype.
In vitro, NOD2 binding has been shown to induce defensin secretion in some epithelial cells

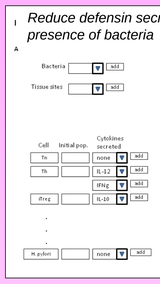
1. Observe *in vivo* level health outcome and *in vitro* level observed mechanism

Impairment of defensin secretion allows pathogenic bacteria to proliferate leading ultimately to hyperinflammation.



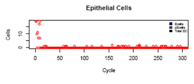
2. Create Revise a biological model to explain tissue-level outcome with mechanism involving in vitro observed mechanism.

Reduce defensin secretion y epithelial cells in the presence of bacteria



3. Hypothesis Test
User specifies ENISI conditions to represent proposed biological model

Observe whether simulated inflammation leads to immunopathogensis.



4. Hypothesis Generation
Model predictions support or reject the hypothesized pathway as a cause of health outcome.
- predict tissue level effects of treatment strategies.
- new hypothesis are generated as alternative pathways are implemented and tested in simulations.

5.Test Model Predictions
*Implement treatments predicted to be successful
*Test for the presence of alternative pathways predicted by simulations

Application

ENISI output for immune response to commensal bacteria (Figure 3) and commensal bacteria in the presence of pathogenic B. hyodysenteria (Figure 4)

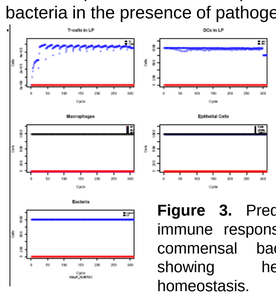


Figure 3. Predicted immune response to commensal bacteria showing healthy homeostasis.

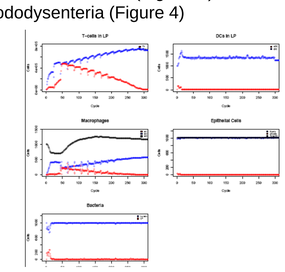
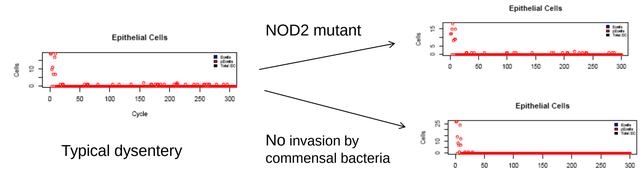
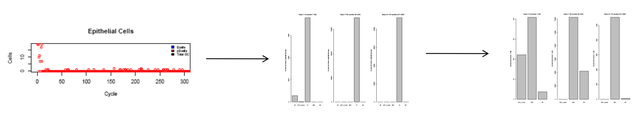


Figure 4. Predicted immune response to *Brachyspira hyodysenteriae* showing transient dysentery marker so epithelial damage and bacterial invasion followed by recovery with micro epithelial damage.

- **Test** plausibility of *in vitro* observed behavior as explanations for observations in *vivo /in situ.*



- **Identify** specific pathways not observable in in vivo experimental systems.



What is the main pathway of residual epithelial damage?

Th1/Th17 is main contributor to epithelial damage

M2 differentiating to M1 is main contributor to Th1/Th17 activation

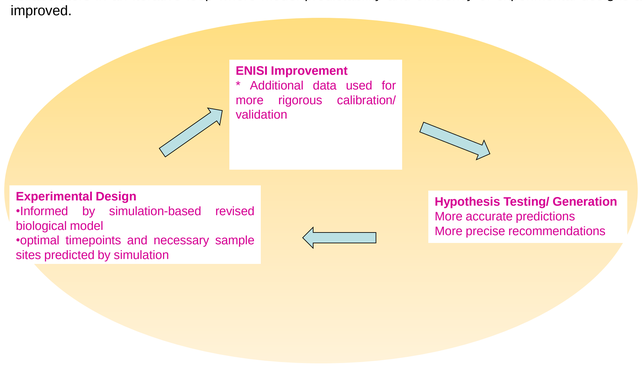
- **Conduct** low-cost, preliminary experiments of proposed interventions/ treatments.
- *Inhibition of M1-mediated Th1/Th17 stimulation will allow clearance of pathogen without residual epithelial damage.*

- **Propose** behaviors not yet tested *in vitro* that could be plausible explanations for observations at the tissue level.
- *Could direct activation of nTreg by commensal bacteria account for its association with lower inflammation?*

Continued Development

The Symbiosis of Simulator Development and Experimental Design

Development of ENISI and refinement of its base model is an ongoing process. This is done with experimental collaborators in an iterative loop where model predictability and efficiency of experimental designs are continually improved.



Additions Underway

- Addition of a Th17 phenotype
- Additional tissue sites including Lymph node
- Additional specific bacterial strains (H. pylori)

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