ENteric Immunity SImulator: A Tool for *in silico* Study of Mucosal Immunity

Keith Bisset, Josep Bassaganya-Riera, Adria Carbo, Stefan Hoops, Raquel Hontecillas, Madhav Marathe, Yongguo Mei, Katherine Wendelsdorf, Dawen Xie, and Jae-Seung Yeom

Network Dynamics and Simulation Science Laboratory (ndssl.vbi.vt.edu), Nutritional Immunology and Molecular Medicine Laboratory (nimml.org), Center for Modeling Immunity to Enteric Pathogens (modelingimmunity.org)
Virginia Bioinformatics Institute, Virginia Tech, Blacksburg VA

ENISI: A simulator of mucosal inflammatory response

ENISI is 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 bacteria and foreign pathogen. ENISI represents a subset of the relevant sites and cells of the gut mucosa. Including:

Cell-types
- Naive T-cell
- Memory T-cell: Central , Effector
- Activated T-cells: Th1, Th17, Induced regulatory (iTreg)
- Natural T-regulatory cells (nTreg):
- Macrophages: Undifferentiated (M0), Inflammatory (M1), Regulatory (M2)
- Dendritic Cells: Immature (iDC), Effector (eDC), Tolerogenic (tDC)
- Epithelial cells: healthy, pro-inflammatory
- Bacteria: inflammatory strains, tolerogenic strains, commensal strain

States
Resting
Active- inflammatory
Active-regulatory
Dead/Anergic
Damaged/impaired

Tissue Sites
Lumen
Lamina Propria (LP)
Lymph Node (LN)

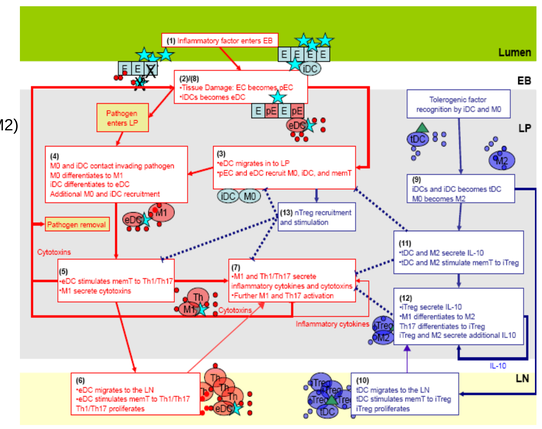

Fig. 1 Illustration of sequential events in the inflammatory (red arrows) and regulatory (blue arrows) pathways that ENISI includes. Dashed lines indicate events that inhibit the occurrence of another event.

The Model

ENISI encodes each immune pathway as an agent-based model representing each individual cell that participates in each component event which may be one of seven cell-types: a **bacteria, epithelial cell, 'sampling' dendritic cell, LP dendritic cell, macrophage, T-helper cell,** or an **nTreg** and occupy a state. These individuals migrate among tissue sites forming a contact network. Each immune event (differentiation, migration, cell death/damage) is represented by contact-dependent or time-dependent state change of an individual cell according to cell-specific rules.

Figure 2 depicts the ENISI model as a network created using CellPublisher. The network portrays inflammatory and regulatory immune cell interactions with epithelial cells and bacteria in the four tissue compartments; lumen, epithelium, lamina propria, and lymph node. A fully annotated, interactive version is publicly available at http://www.modelingimmunity.org/modeling/enisi/.

Users can navigate through different cells types and interactions on the network and get useful information though the info windows.

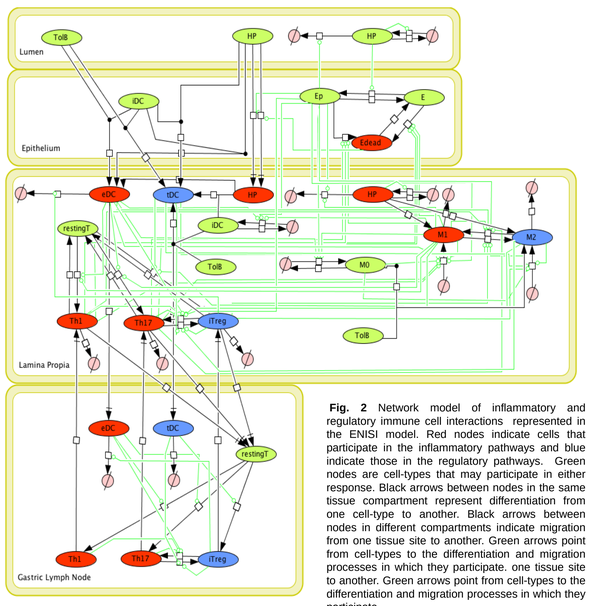

Fig. 2 Network model of inflammatory and regulatory immune cell interactions represented in the ENISI model. Red nodes indicate cells that participate in the inflammatory pathways and blue indicate those in the regulatory pathways. Green nodes are cell-types that may participate in either response. Black arrows between nodes in the same tissue compartment represent differentiation from one cell-type to another. Black arrows between nodes in different compartments indicate migration from one tissue site to another. Green arrows point from cell-types to the differentiation and migration processes in which they participate. one tissue site to another. Green arrows point from cell-types to the differentiation and migration processes in which they participate.

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, it is appropriate to testing and generating hypothesis. 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.

Simulation results are automatically provided as plots of cell population counts over time of the simulated infection (Figure 3) in each tissue site or as a tissue-specific heat map showing the relative level of different cell phenotypes in each tissue compartment over the course of infection (Figure 4). Figure 3 gives an example of changes in *in silico* T cell dynamics in the LP observed under different experimental scenarios executed in ENISI. Such results are given as plots of

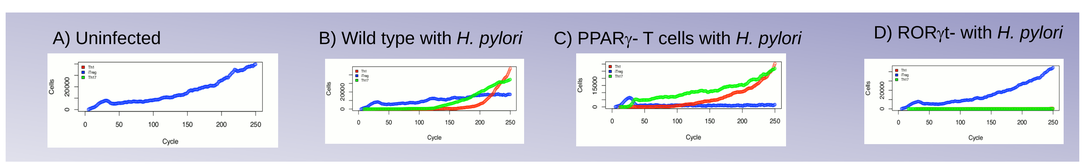

Fig. 3 Active T cell populations in the LP over a simulated 2 month infection with pathogenic *H. pylori* under different conditions. A) non-infected, B) Infected wildtype host, C) Infected host with a PPARγ knockout in T cells, and D) Infected host with a RORγt knockout.

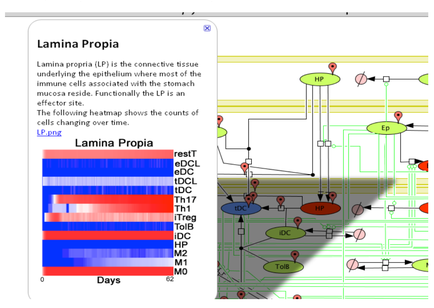

Fig. 4 Sample results from a single instance of a simulated pathogenic *H. pylori* infection as a heat map of cell types present in the LP where the x-axis is the time point post-infection and the y-axis is labeled with cell-types. For each cell-type at each timepoint the color indicates the cell-type count in the tissue compartment. Red indicates the highest values for counts overall cell-types, blue indicates the lowest, and white is a count in between these lowest and highest values. Here we can see that in early infection the highest counts belong to resting and tolerogenic immune cells with inflammatory Th1, Th17, and M1 increasing towards the end of the simulated infection period. The results are presented in the context of the fully annotated network model using the enhanced version of CellPublisher in SBML.

Study: Functions of *H. pylori* strain 26695 pathogenicity

With ENISI one may analyze an infection recreated *in silico* to inform hypothesis for the sequence of events occurring in a real infection. As an example, we simulate infection of individual mice with the cpi-containing H. pylori strain 26695 carried out by experimental collaborators.

The *H. pylori strain* 26695 was represented by assigning functions to **commensal bacteria** based on experimental observations of *in vivo* infections conducted by collaborators. Infection was simulated by adding *H. pylori* on day 2 and following the state changes and migration of cells over 63 days .Figure 5 shows the dynamics of specific cell populations in 7 replicate infections over time in a *in silico* tissue sample representing roughly 1% of the murine gastric mucosa and reflects experimental observations of *in vivo* mice infections: *i) H. pylori*-specific immune response is nearly undetectable before day 30 p.i., *ii)* by day 60, not prior, there is a statistically significant increase in M2 macrophages in the infected group over the control group, but not an increase in M1, *iii)* by day 60 p.i., but not prior, there is a statistically significant increase in both effector and tolerogenic dendritic cells in the GLN, *iv)* by day 60 p.i. there is a significant increase in active T cells in the LP in the infected group, with Th1 dominating Th17 on average.

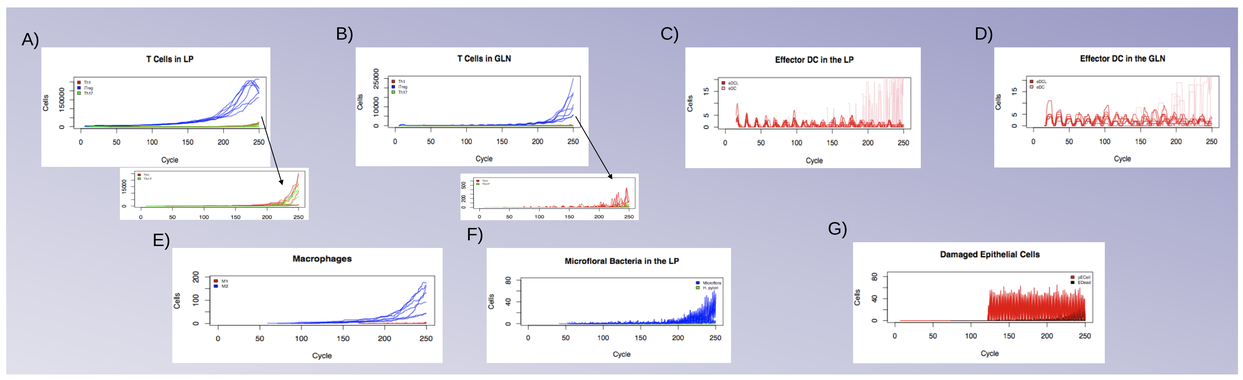

Fig. 5 Response to *H. pylori 26695* in 7 individuals A) Active T cell populations in the LP, B) Active T cells in the LN, C) Effector dendritic cells (eDC) in the LP, D) eDC in the LN, E. Active macrophages, F) microfloral bacteria in the LP, G) Damaged and dead epithelial cells

It can be seen that the increase in immune activity is associated with mounting epithelial damage, (Figure 5G) represented by transition of an individual epithelial cell from the healthy (Ecell) state to the pro-inflammatory (pECell) state and from the pECell state to the Edead state. To identify the pathways by which this mounting immune response is associated with tissue damage we analyze the contact network formed during the simulation replicate that resulted in the greatest epithelial damage (replicate 5).

Cause of EC damage: Th1-secreted factors

Figure 6 is a histogram of number of individuals in each phenotype that induce state transition EC ell → pECell showing that in the second month, when most damage is seen, Th1 is the main inducer of epithelial damage.

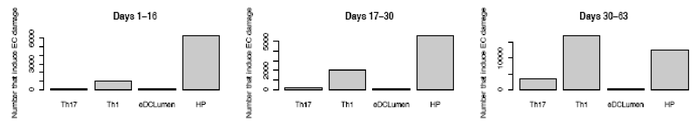

Fig. 6 Number of individuals in each state that induce state transition of epithelial cells from health to damaged, EC -> pECell.

Cause of mounting Th1 levels: resting T cell recruitment and subsequent stimulation by eDC

It was found that T cells stimulated to Th1 contacted only 'sampling' eDC stimulated in the lumen. Figure 7 shows that the level of eDC over the course of infection remains constant (Fig. 7A and 7B) where as the number of resting T cells in the LP (Fig. 7C) due to recruitment.

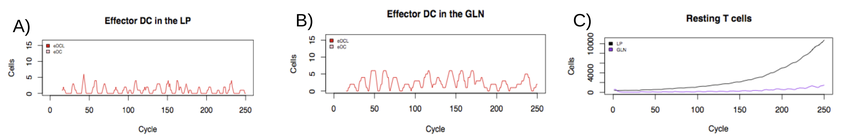

Fig. 7. Cell populations over course of 2 month infection in replicate 5. A) eDC in the LP, B) eDC in the LN, C) resting T cells

Factors contributing most to resting T cell recruitment: Pro-inflammatory epithelial cells and eDC
Analysis of the contact network indicated that both pro-inflammatory epithelial cells (pECell) and 'sampling' effector DC (eDCL) appear to be equally responsible for inducing state transition Tsource -> restingT, representing T cell recruitment.

Conclusion: *H. pylori* 26695 induced tissue damage in first two months of infection is mediated by stimulation of 'sampling' dendritic cells of the lumen to effector phenotypes that recruit and subsequently stimulated resting T-helper cells to a Th1 phenotype. Notably it does not involve invasion of H. pylori in to the LP nor macrophage activation, though it is associated these an increased frequency of these events.

Cyberinfrastructure for ENISI

The ENISI tool requires an extensive CyberInfrastructure as depicted in Figure 8. The features of this CyberInfrastructure include:

- **Multiplexing:** Connects User Interfaces, Models, Compute Resources, and Digital Library
- **Resource Allocation:** Assigns models, data, and compute resources to user requests
- **Security:** Data, models, compute resources restricted to authorized users, or publicly available
- **Compartmentalization:** Models and data can be restricted to selected compute resources, only model output available elsewhere
- **Expansion:** User interfaces, data, models, and compute resources can be easily added, all other resources are immediately accessible
- **Deployment:** Supports *geographic* and *organizational* distribution for expanded access to resources

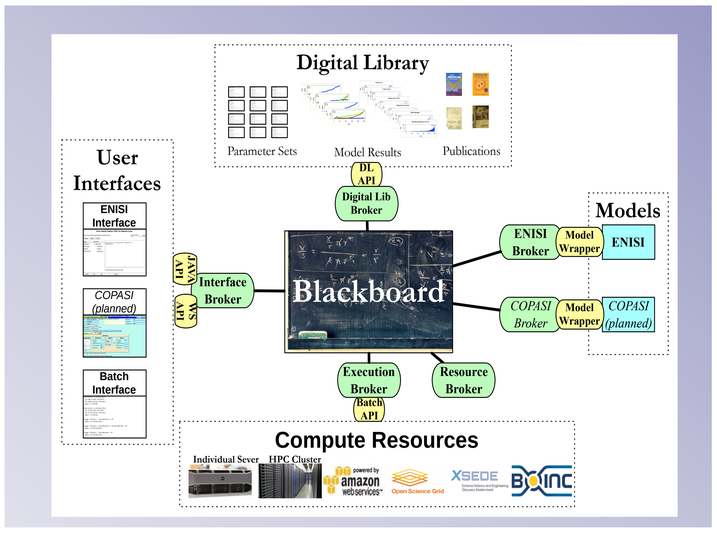

Fig. 8 A scheme of the extensive cyberinfrastructure that lies behind the ENISI tool.

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