

Host immunological conditions that determine status of *Helicobacter pylori* as pathogenic or commensal bacterium

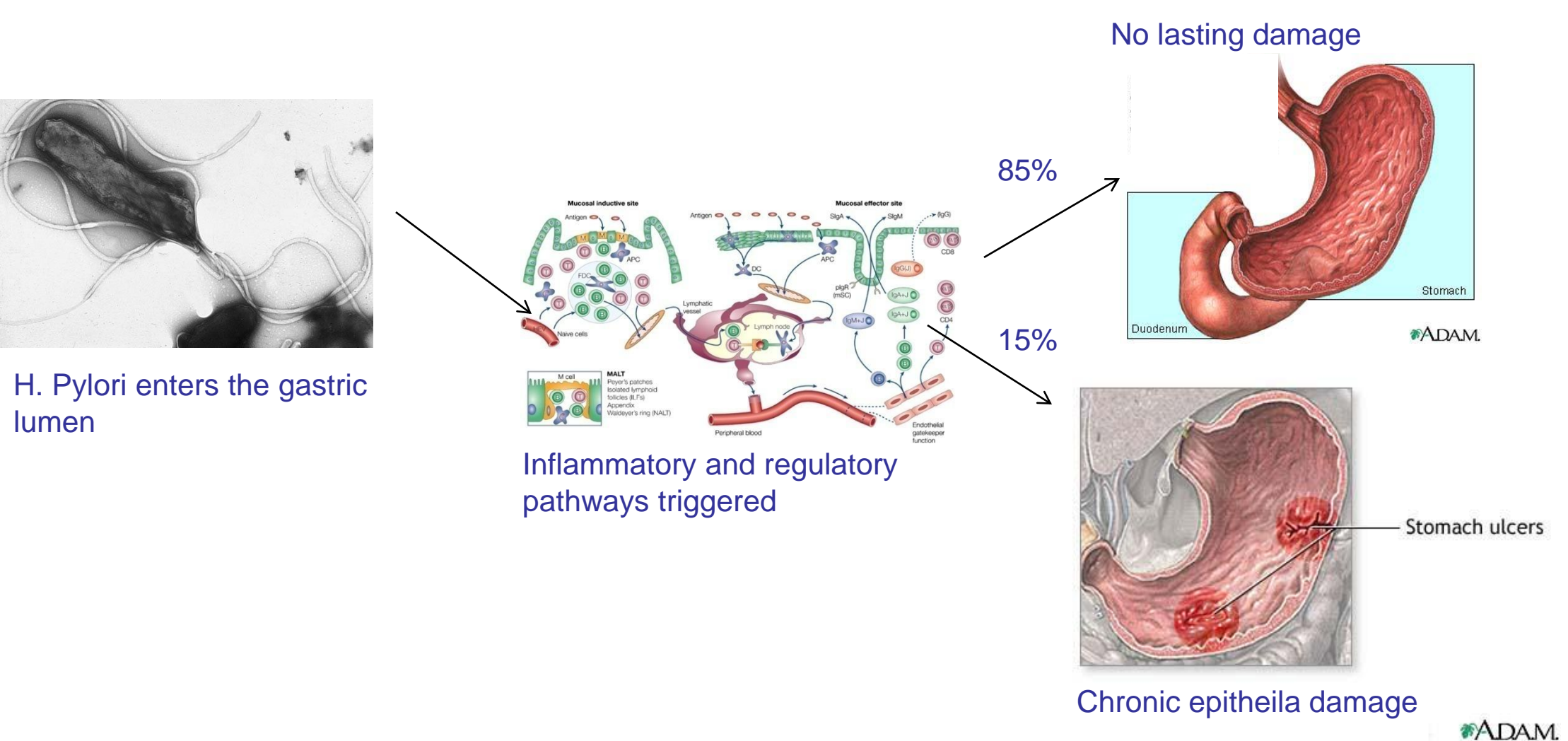
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Helicobacter pylori: Friend and Foe

Inflammatory/Regulatory Response and H. pylori Pathogenesis

In 85% of H. pylori infections the bacteria persists as a commensal bacteria in the gastric mucosa with no negative impact on the host. However, in ~15% of cases the infection is associated with ulcer formation as persistent inflammation induces lesions in the gastric epithelium.

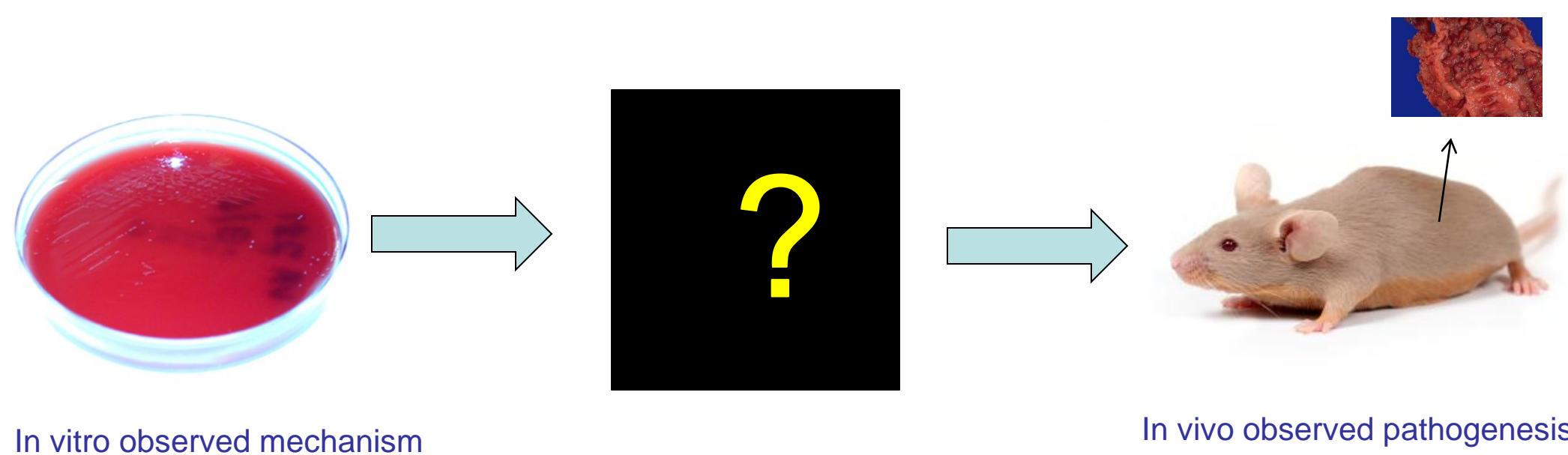


The immune response in the gastric mucosa involves inflammatory and regulatory pathways. Inflammatory/effector cells such as M1, Th1, Th17 secrete factors that i) recruit more immune cells, ii) promote activation to inflammatory phenotypes, and iii) secrete substances that destroy bacteria and damage host tissue indiscriminantly. Regulatory immune cells such as tolerogenic macrophages, dendritic cells, and T-regulatory cells (Treg) act antagonistically to their inflammatory counterparts through various contact dependent mechanisms and secretion of IL-10 and TGF- β , two cytokines that elicit potent anti-inflammatory responses.

Prevailing theories state that individuals with different health outcomes are infected by H. pylori strains with different immunomodulatory capabilities. Some of those proposed include:

- Ability to invade mucous layer gaining access to epithelial barrier
- Expression of various immune factors by epithelial cells (recruitment chemokines, inflammatory cytokines, defensins, etc.)
- Induction of tolerogenic phenotype in antigen-presenting cells, including upregulation of IL-10
- Modification of ability of macrophages to produce NO and other killing factors
- Reduce stimulation of T-cells
- Inhibition of Th proliferation
- Induction of inflammatory Th17 or Th1 response as opposed to iTreg

Many of these capabilities, however, have been identified by observing immune cells in the presence of *H. pylori* *in vitro*. Though these effects undoubtedly mediate the inflammatory response, their net effect in the gut mucosa in the presence of complex cross-reacting inflammatory and regulatory pathways can not be intuitively predicted.



The Question

Given established inflammatory and regulatory pathways of the gut mucosa, which of these immunomodulatory capabilities would likely permit a strain to i) persist in the gastric mucosa with no harm to the epithelial barrier or ii) induce an immunopathogenic inflammatory response resulting in epithelial damage?

The Approach- A Model of Regulatory and Inflammatory Response in Gastric Mucosa

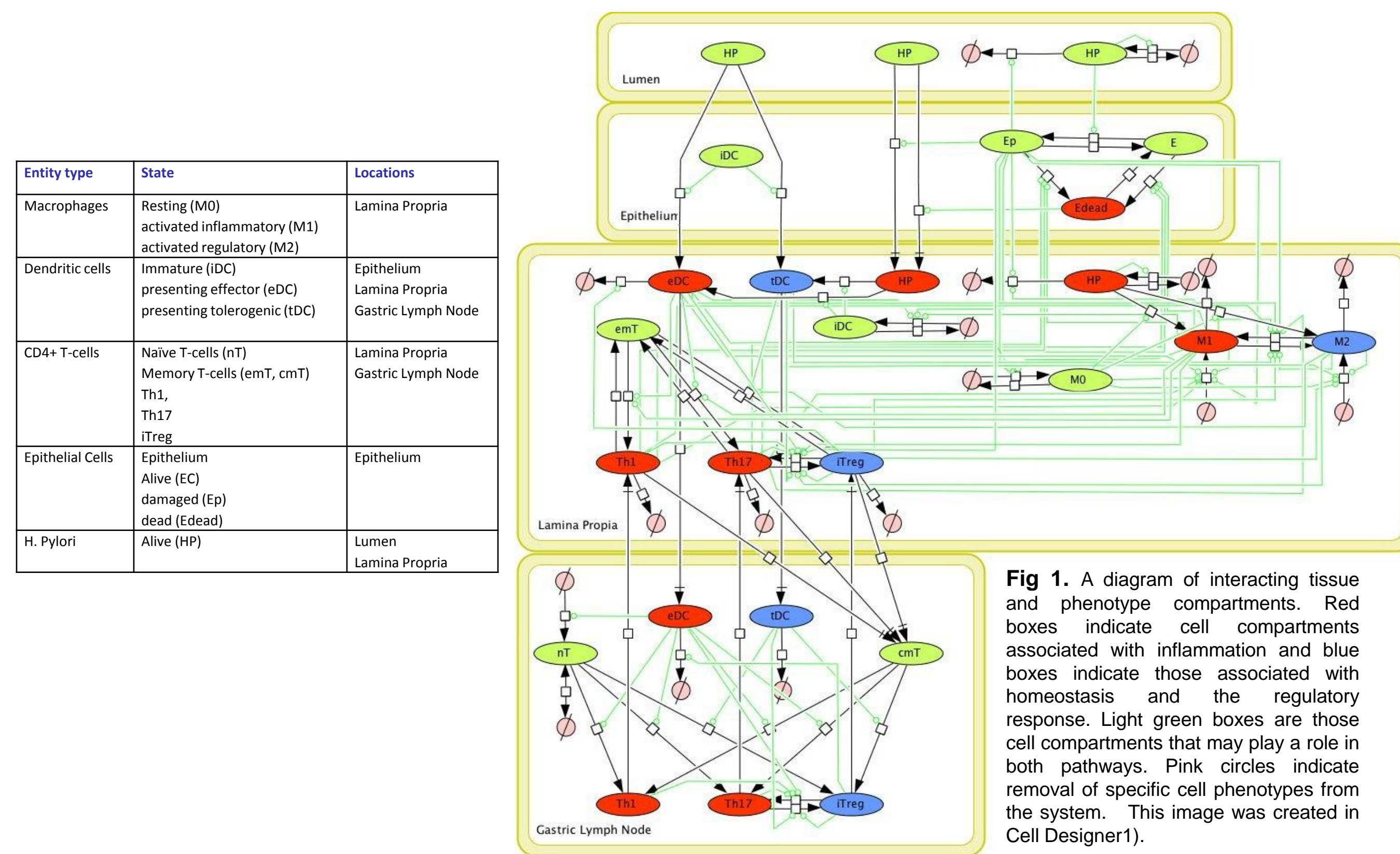
To test hypothesized in vitro observed mechanisms behind in vivo observed immunopathology we create a model representing response of immune cells of the regulatory and inflammatory pathways as well as epithelial cells to H. pylori in the gut mucosa. In this model we include various mechanisms by which H. pylori may affect the host immune response associated with pathogenic vacA+ and cagA+ strains.

We then implement this model and, through simulations, observe the predicted impact of each mechanism on epithelial cell and H. pylori concentrations following the initial inflammatory response. Simulation result indicate which mechanisms play a significant role in establishing a commensal relationship, i.e. full recovery of the epithelial layer along with a low level persistence of H. pylori versus an immunopathogenic response, i.e. the epithelial layer remains chronically reduced.

The Model

The model shown in Fig. 1 consists of 24 variables, 83 constant parameters, and includes four tissue sites of the gastric mucosa: i) lumen, ii) epithelium, iii) lamina propria (LP), and iv) the gastric lymph node (GLN).

Entities are represented as populations of immune cells, epithelial cells, and H. pylori. Immune cell populations are compartmentalized by immunological state (resting, active inflammatory, regulatory), epithelial cells are compartmentalized by health state (normal, damaged, dead). All populations are further compartmentalized by location in one of 4 tissue sites. Cell differentiation is represented as flow from one cell-type to another and migration as flow from one location compartment to another as depicted in the scheme below.



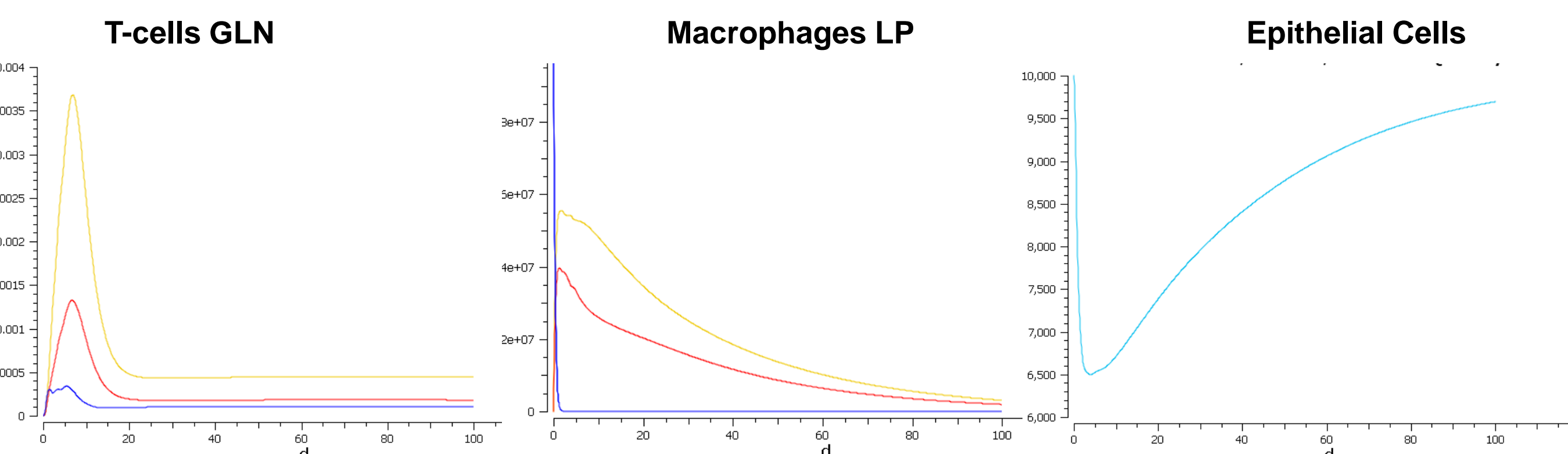
Each immunomodulatory mechanisms found in the literature is included as a reaction that is governed by a specific parameter as shown in Table 2 below

Parameter(s)	Immunomodulatory mechanisms
KEB	Invasion of mucous layer and contact with epithelial cells.
Br	Secretion of chemoattractant s (IL-8) by epithelial cells following contact with H. pylori.
Bc	Secretion of inflammatory factors that induce M2->M1 and iTreg->Th17 by epithelial cells following contact with H. pylori.
Bd	Secretion of defensins by epithelial cells following contact with H. pylori.
Bp	Induction of cytoskeletal changes in epithelial cells following contact with H. pylori.
vHP	Induction of effector and M1 response in dendritic cells and macrophages, respectively.
uM1	Induction of cytotoxin expression (NO) by macrophages.
m_HP	Inhibition of Th proliferation
ath1	Stimulation of a naïve T-cell to Th1
ath17	Stimulation of a naïve T-cell to Th17
atreg	Stimulation of a naïve T-cell to iTreg

Table 2. Immunomodulatory mechanisms included in the model.

Timecourse Predictions

The model was implemented in COPASI (2). Shown below are predicted timecourses for sample cell populations following in silico inoculation of H. pylori in the lumen of a naïve host at a concentration of 10E10/mL. The qualitative dynamics follow those expected with 2 distinct phases: acute inflammation followed by recovery (3).



Full recovery is then defined as replenishment of alive epithelial cells in the post-inflammation phase to pre-inflammation levels, i.e. $E(t=0) = E(t=100)$. An ulcer or pathogenic response would be a case in which epithelial cell concentration post-inflammation remains reduced, i.e. $E(t=0) > E(t=100)$.

Initial Insights:

Determinants of recovery vs. ulcer

To assess the potential role of strain-specific immunomodulatory mechanisms in determining a commensal vs. pathogenic relationship between H. pylori and the host, we conducted a local sensitivity analysis of all variable concentrations in the post-inflammation phase to all parameter values including those listed in Table 2. In this manner we observed the net effect of each inflammatory/ regulatory mechanism on epithelial cell recovery (marked by $E(t=100)$) and H. pylori persistence (marked by $HP(t=100)$).

Local SA Results and Implications

Relative changes in $E(t=100)$ and $HP(t=100)$ given varying values of model parameters indicated the following relationships:

HP survival in lumen is not significantly impacted by immunomodulatory functions and is strikingly resilient to any immune response in the underlying LP

•The only parameters that significantly affect HP in the lumen are those that deal with inherent biology (birth and death rates) and those that directly govern the level of iDC in the epithelium (Not shown).

HP clearance from the LP is lead by phagocytosis by iDC as opposed to M0 and defensin-secreting Epithelial Cells.

•HP in the LP is negatively correlated with factors directly governing rate of removal by various immune cells, specifically iDC more than others M0 and Epithelial cells.

Strains that inhibit NO secretion by M1 are most likely to have a commensal relationship.

•In general, E and HP in the LP at day 100 are negatively impacted by all parameters associated with increased M1 creation. Specifically, uM1 is the parameter most positively associated with Edead at day 100 (Table 3).

Whether infection results in acute inflammation with recovery or chronic lesion formation is not determined by strain-specific effects on epithelial cells, but rather on macrophages.

•E is not significantly affected by any strain-specific parameters Bc, Bp, Bd, and Br (Table 3).

Induction of regulatory and inflammatory T-cell phenotypes in the GLN ultimately leads to inflammation induced epithelial damage.

•Edead is positively correlated with ath1, ath17, and atreg (Table 3). This is due to fact that predicted conditions favor a iTreg -> Th17 transition (simulation results not shown).

	vHP	atreg	ath1	ath17	Bc	Bp	uM1	Bd	Br	m_HP
HP in the Lumen	-3.6E-09	-3.9E-08	-8.0E-09	-2.4E-08	3.3E-11	3.3E-12	-2.2E-06	-1.8E-12	2.4E-12	5.2E-12
HP in the LP	-3.3E-05	-4.0E-04	-8.2E-05	-2.4E-04	5.5E-08	4.5E-08	-2.3E-02	3.0E-08	3.3E-08	6.5E-08
E	-4.4E-05	-5.3E-04	-1.1E-04	-3.2E-04	7.3E-08	5.9E-08	-3.0E-02	4.0E-08	4.5E-08	8.6E-08
Edead	1.4E-03	1.7E-02	3.5E-03	1.0E-02	-2.3E-06	-1.9E-06	9.7E-01	-1.3E-06	-1.4E-06	-2.7E-06

Table 3. Relative change in H. pylori and Epithelial cell populations on day 100 to parameters listed in Table 2. Those below 1e-04 are not considered significant.

Future Directions: A tool for hypothesis testing and generation

Inform experimental design

- Predict optimal datapoints to capture critical immune response dynamics
- Gather experimental timecourse data to calibrate model for quantitative predictions of health outcomes

Indicate plausible mechanisms of varying clinical outcomes

- Test hypothesis for association between strain-specific clinical outcomes and specific in vitro-identified immunomodulatory mechanisms
- VacA is associated with pathogenesis in humans, but not in mice. Simulations allow one to add aspects of host immunity that are proposed to account for difference in mouse/human susceptibility and observe which determine the difference between a pathogenic/non-pathogenic infection.

Generate hypothesis for novel immunomodulatory mechanisms

- Test hypothesis for immune cells dynamics observed in experimental animal models using different strains.



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