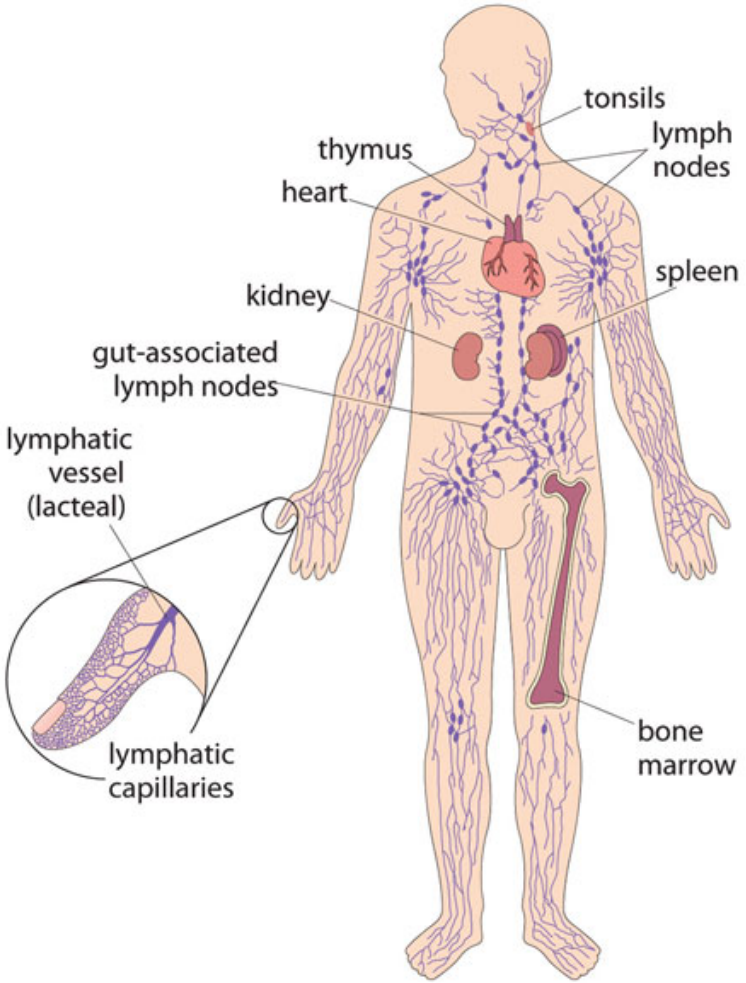
Towards a Virtual Immune System: Multi-Scale Modeling of CD4+ T Lymphocytes

Presenting author: Kenneth Y. Wertheim, PhD

Authors: Kenneth Wertheim, Bhanwar Lal Puniya, Alyssa La Fleur, Ab Rauf Shah, Matteo Barberis, and Tomas Helikar

Travel fellowship funding generously provided by SysMod COSI.

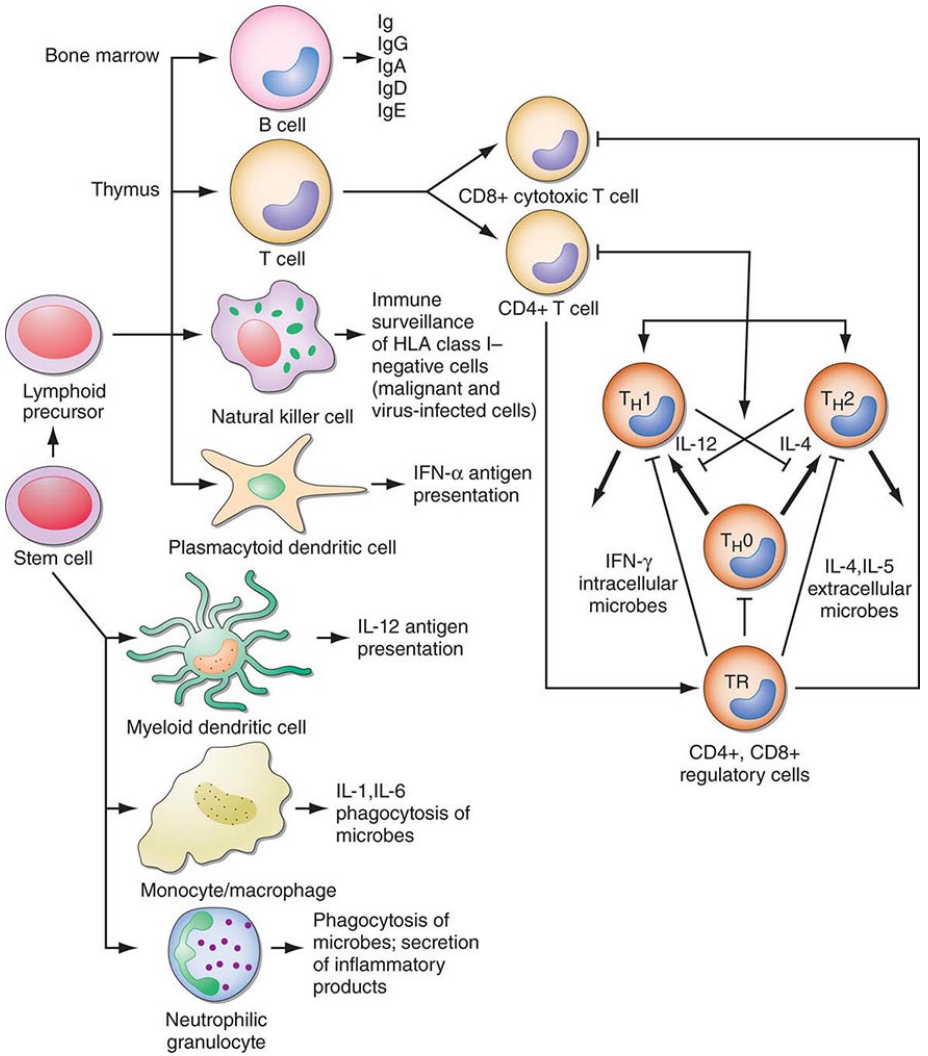
The Immune System



- Defends the body against disease-causing invaders.
- Whole-body system: many tissues and organs.
- Immunodeficiency such as AIDS caused by HIV.
- Autoimmunity such as celiac disease.
- Spans many scales of biological organization.

Source: The Open University, SXR376 Preparatory Reading, fig. 1.2, page 7. http://moodle.digital-campus.org/mod/page/view.php?id=18790.

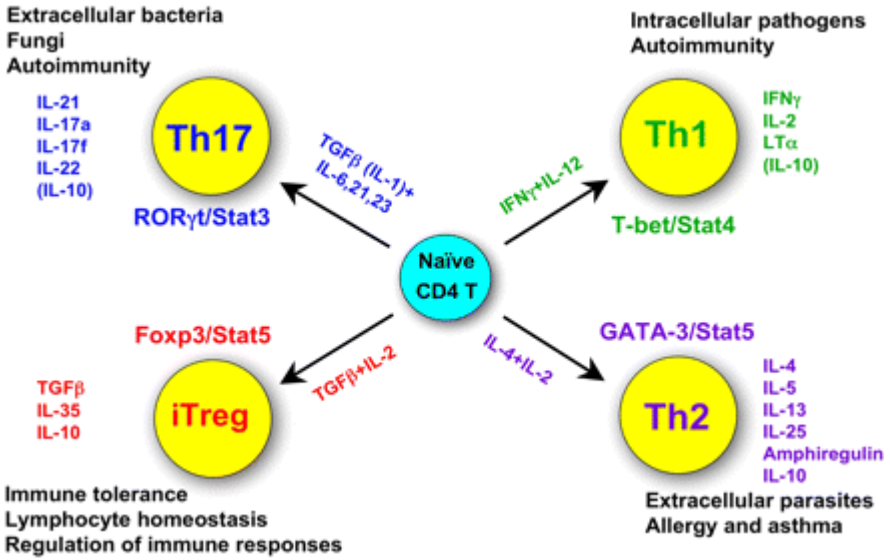
Network of Molecules and Cells



Source: https://clinicalgate.com/introduction-to-the-immune-system-2/#ch372efig2.

- Mathematically and computationally capture the information flow in the network.
- Cell population: different cell types, phenotypes, and metabolic rates.
- Extracellular: concentration dynamics of cytokines and antibodies.
- Intracellular: signaling, gene regulation, and metabolism.
- Our focus: systems-level dynamics between these scales.
- Example: CD4+ T lymphocytes.

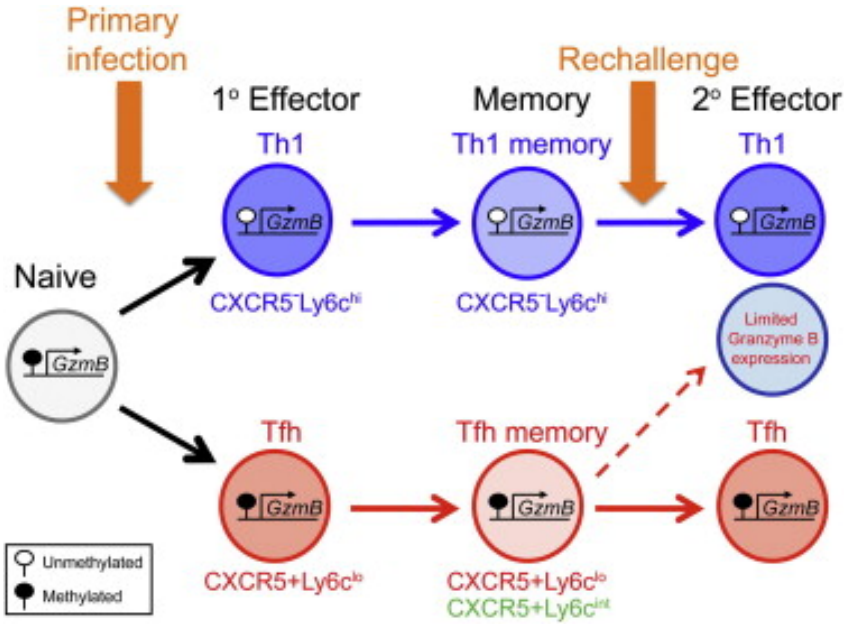
CD4+ T Lymphocytes



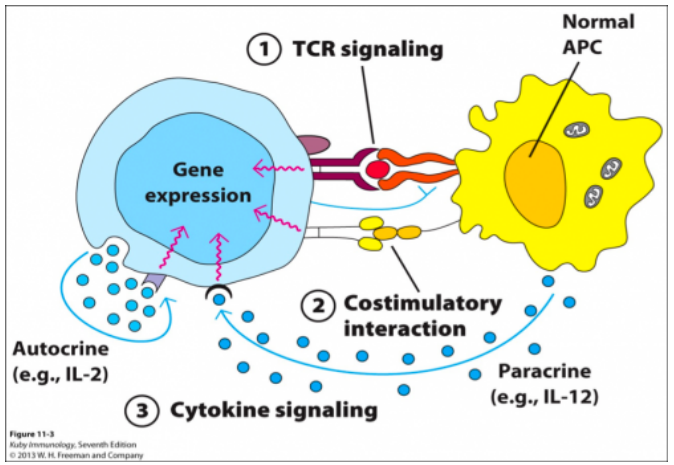
Source: Zhu, J. and Paul, W. E. (2008). CD4 T cells: fates, functions, and faults. *Blood*, 112(5), 1557-1569. https://doi.org/10.1182/blood-2008-05-078154.

- Central to the adaptive immune system: antigen-specific, slow-forming, and long-term.
- They secrete cytokines to unleash other immune cell types, *e.g.*, CD8+ T lymphocytes, macrophages, *etc*.
- Different phenotypes, different purposes.

CD4+ T Lymphocytes



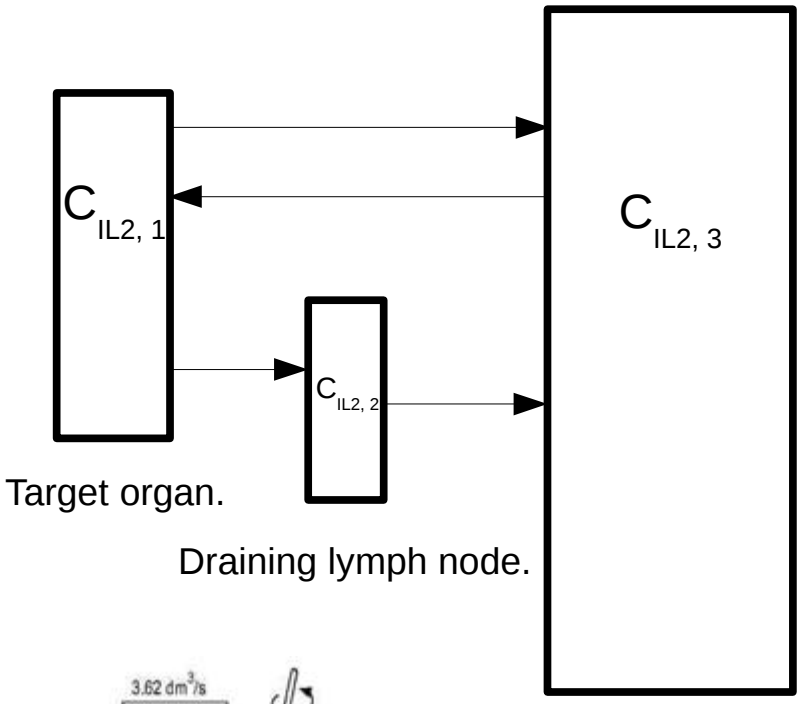
Source: Hale, J. S., *et al*. (2013). Distinct memory CD4+ T cells with commitment to T follicular helper-and T helper 1-cell lineages are generated after acute viral infection. *Immunity*, 38(4), 805-817. https://www.sciencedirect.com/science/article/pii/S1074761313001428?via%3Dihub.

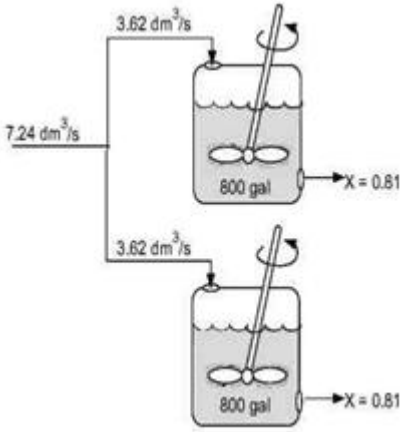


Source: Owen, J. A., *et al*. (2013). *Kuby immunology*. 7th. New York: WH Freeman, 27(692), 109.

- Naive cells activate in response to an infection.
- Effector cells divide rapidly and produce cytokines.
- Memory cells are the effector cells that outlive the infection.
- Memory cells respond to subsequent infections faster and more strongly than naive cells.

Model Architecture: Extracellular

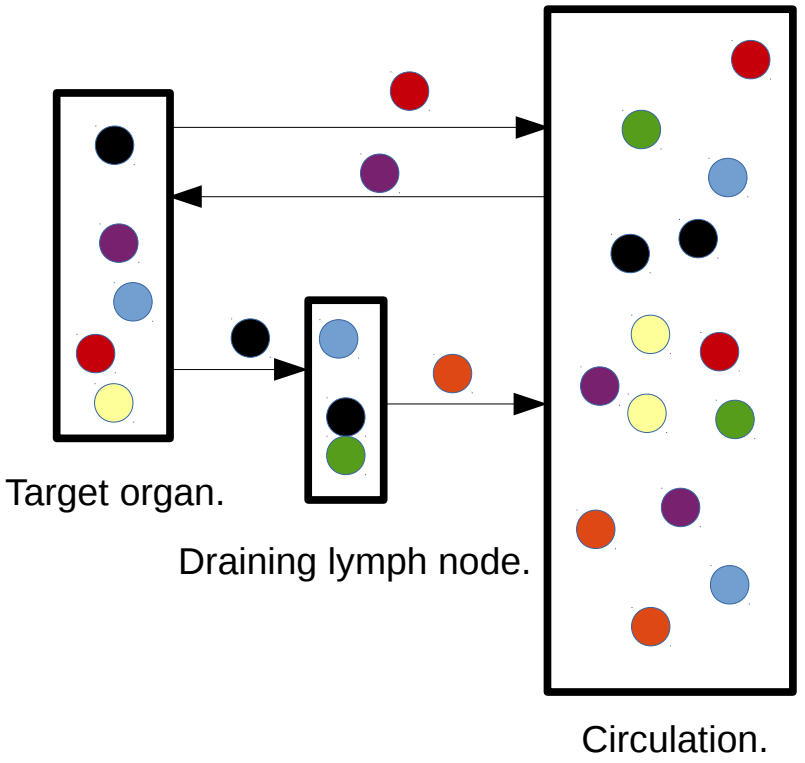


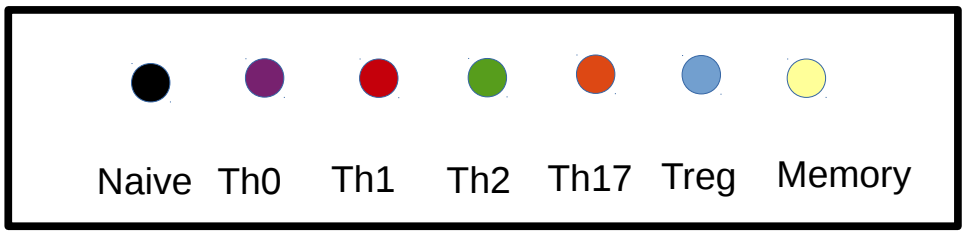


- Extracellular: 11 cytokines (IL2, IL4, IL6, IL12, IL17, IL18, IL21, IL23, IL27, IFNg, and TGFb).
- Three spatially lumped concentrations.
- Functions of time only.
- Three continuous stirred-tank reactors.
- Two downstream units in parallel.
- With a recycle stream.

Source: Fogler, H. S. (2010). *Essentials of chemical reaction engineering solution manual*. Pearson Education. https://www.chegg.com/homework-help/asked-explore-example-problems-chapter-learn-effects-varyin-chapter-5-problem-2qp-solution-9786612872860-exc.

Model Architecture: Cell Population





- Cell population: CD4+ T lymphocytes (naive, Th0, Th1, Th2, Th17, Treg, and memory).
- Move between the compartments; different migration patterns in health and during infections.
- Sense and produce cytokines.
- Agent-based model.
- The invading antigen and the other immune cells are abstracted into a user-defined input signal.

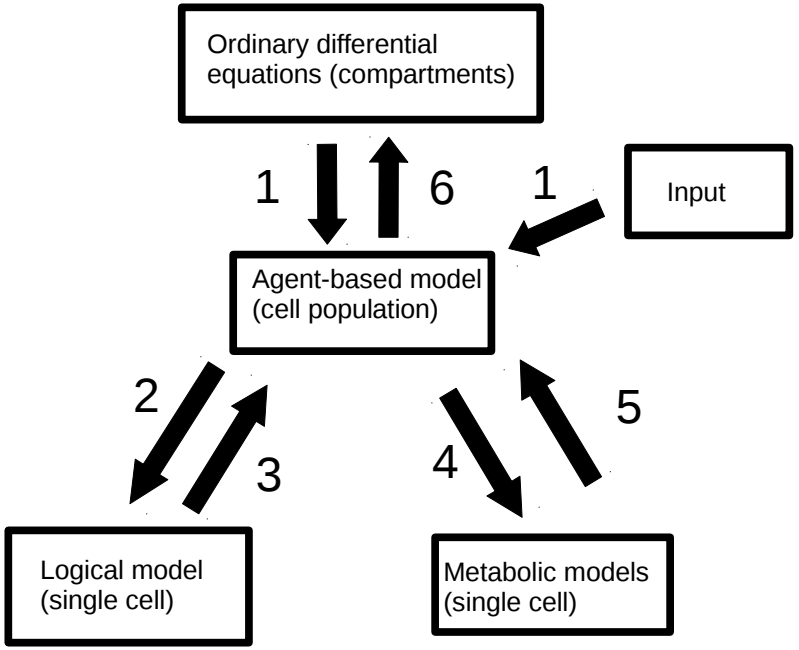
Model Architecture: Information Flow

Production due to infections and the explicitly modeled immune cells.

$$\frac{dC}{dt} = P - k_{deg}C + N_{in}P_{in} + N_{ex}P_{ex} + Convection$$

Natural production and degradation.

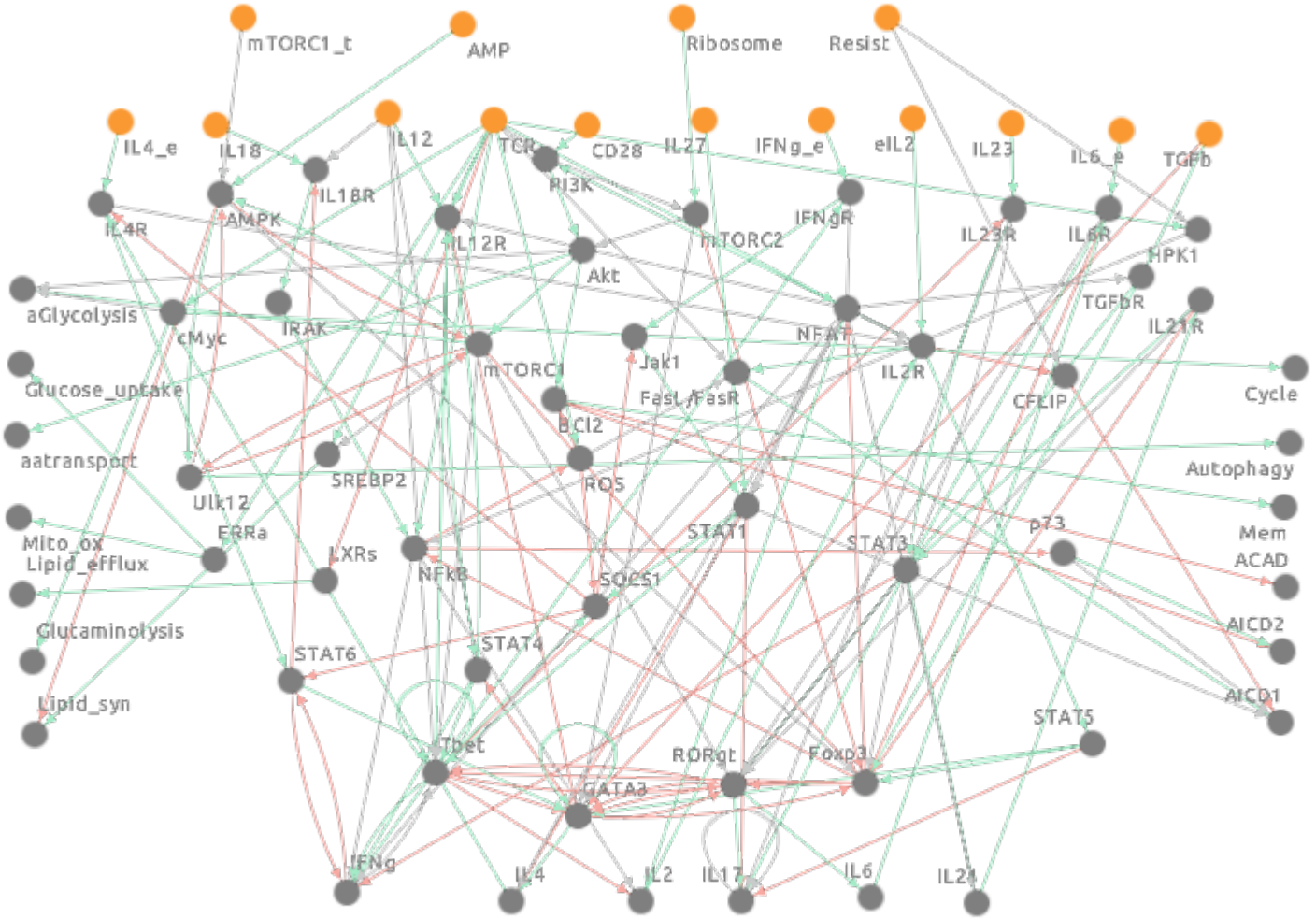
Movement between the three compartments.



- For each cytokine, there are three such equations.
- Coupled through the convection terms.
- Linear reaction terms. Dynamics of species A do not affect species B.

- Agent-based model is parametrized by the concentrations and user-defined input.
- One logical model and five metabolic models in each agent.
- Agent-based model parametrizes the ordinary differential equations.

Signaling and Gene Regulation

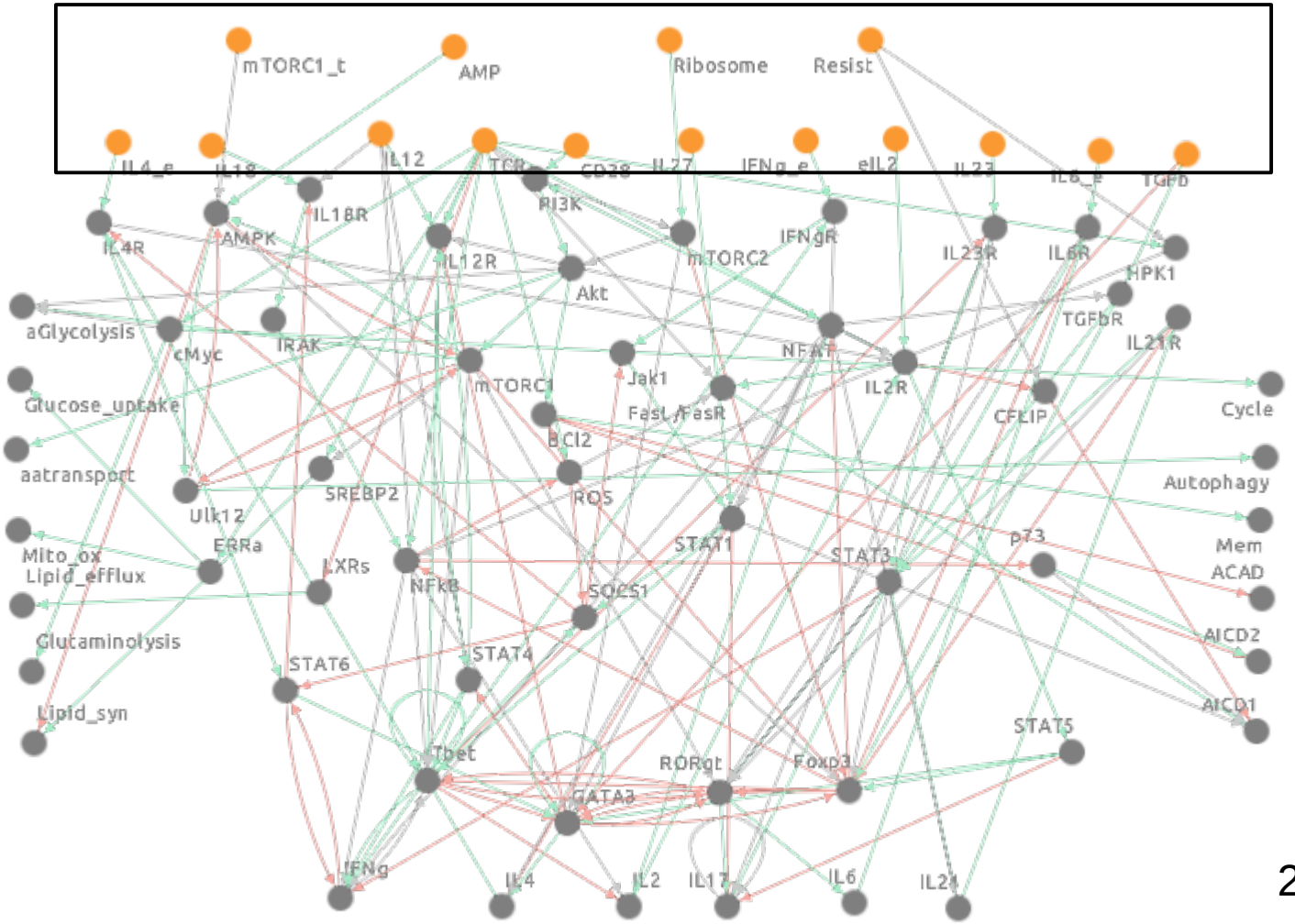


- Logical model.
- Each node is a Boolean variable (0 or 1).
- Yellow nodes: inputs, stochastically on or off.
- Grey nodes: outputs, determined by the states of all nodes.
- Markov chain.
- Activity level of an output node: fraction of iterations where it is on.
- 73 nodes (15 input nodes) and 156 edges.

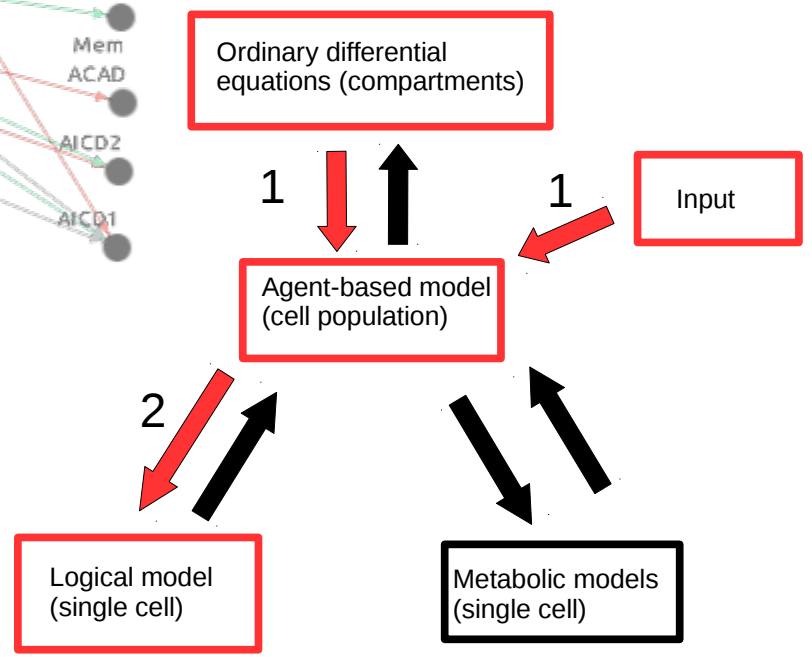
Model source: Puniya, B. L., *et al.* (2018). A mechanistic computational model reveals that plasticity of CD4+ T cell differentiation is a function of cytokine composition and dosage. *Frontiers in Physiology*, 9, 462.

Cell Collective: Helikar, T., *et al.* (2012). The cell collective: toward an open and collaborative approach to systems biology. *BMC systems biology*, 6(1), 96.

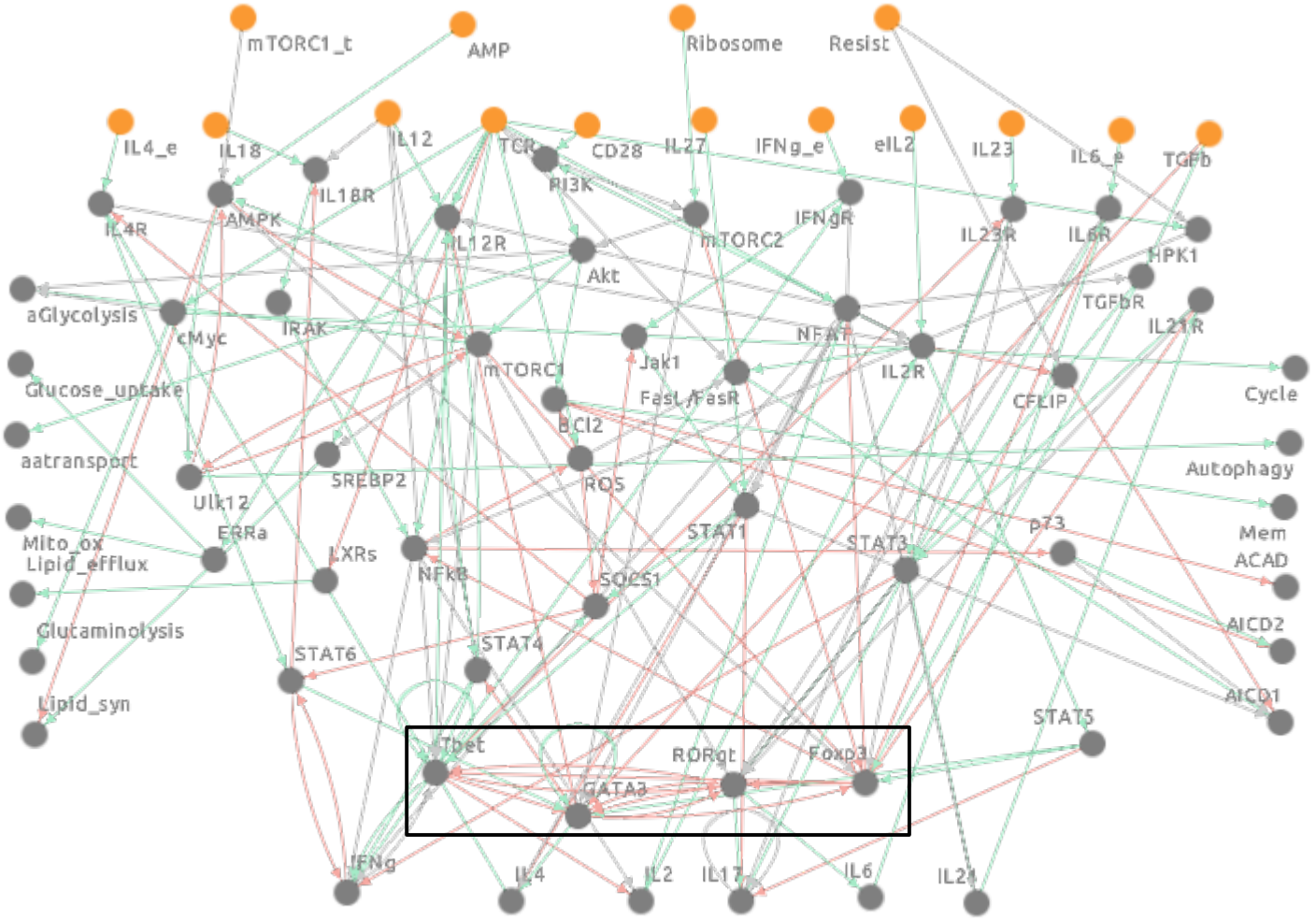
Signaling and Gene Regulation



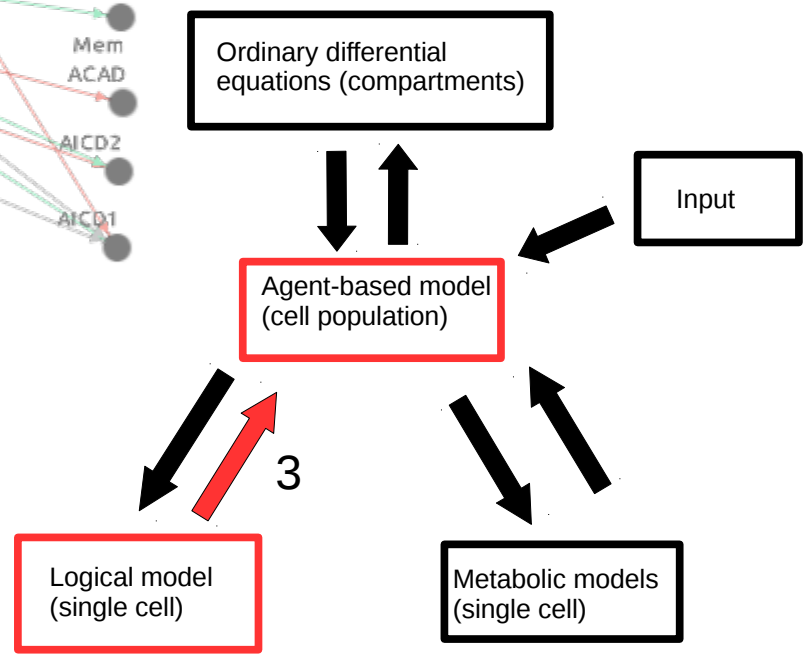
- Three types of inputs
- User-defined input, roughly corresponding to the antigen load.
- Cytokine concentrations.
- Agent attributes *e.g.* division count and AMP/ATP ratio.



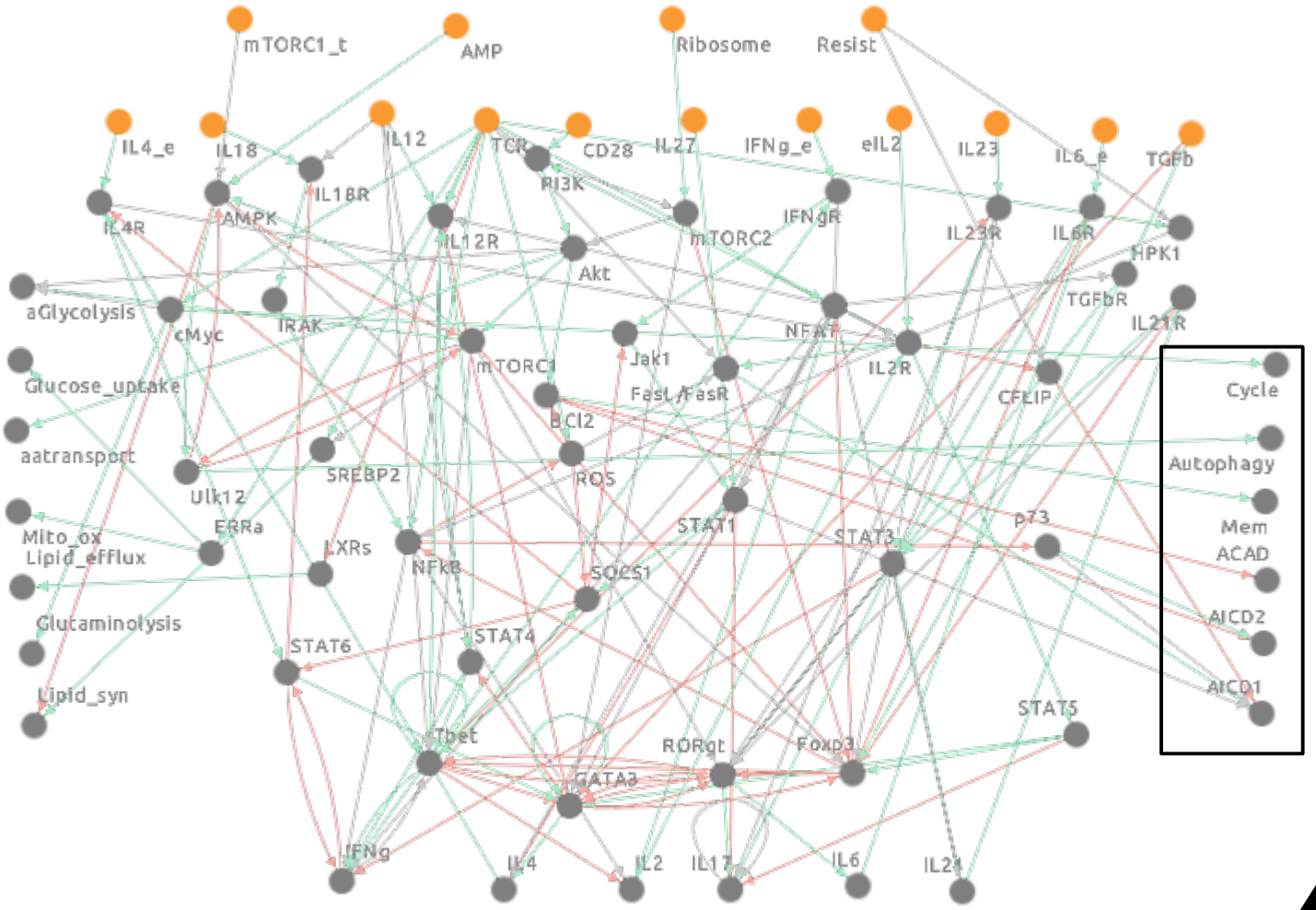
Signaling and Gene Regulation



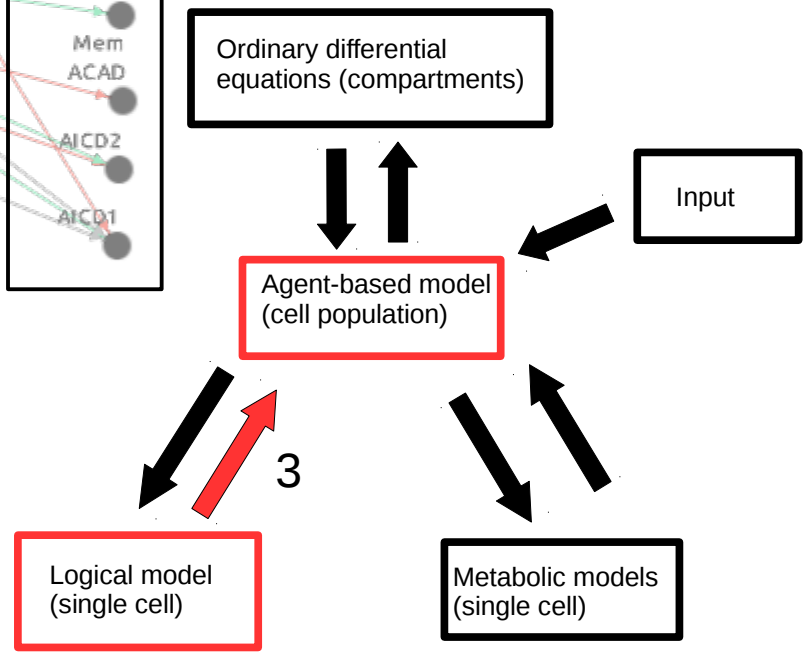
- Four transcription factors.
- Tbet for Th1, GATA3 for Th2, RORgt for Th17, Foxp3 for Treg.
- Activity levels of these nodes become agent attributes.



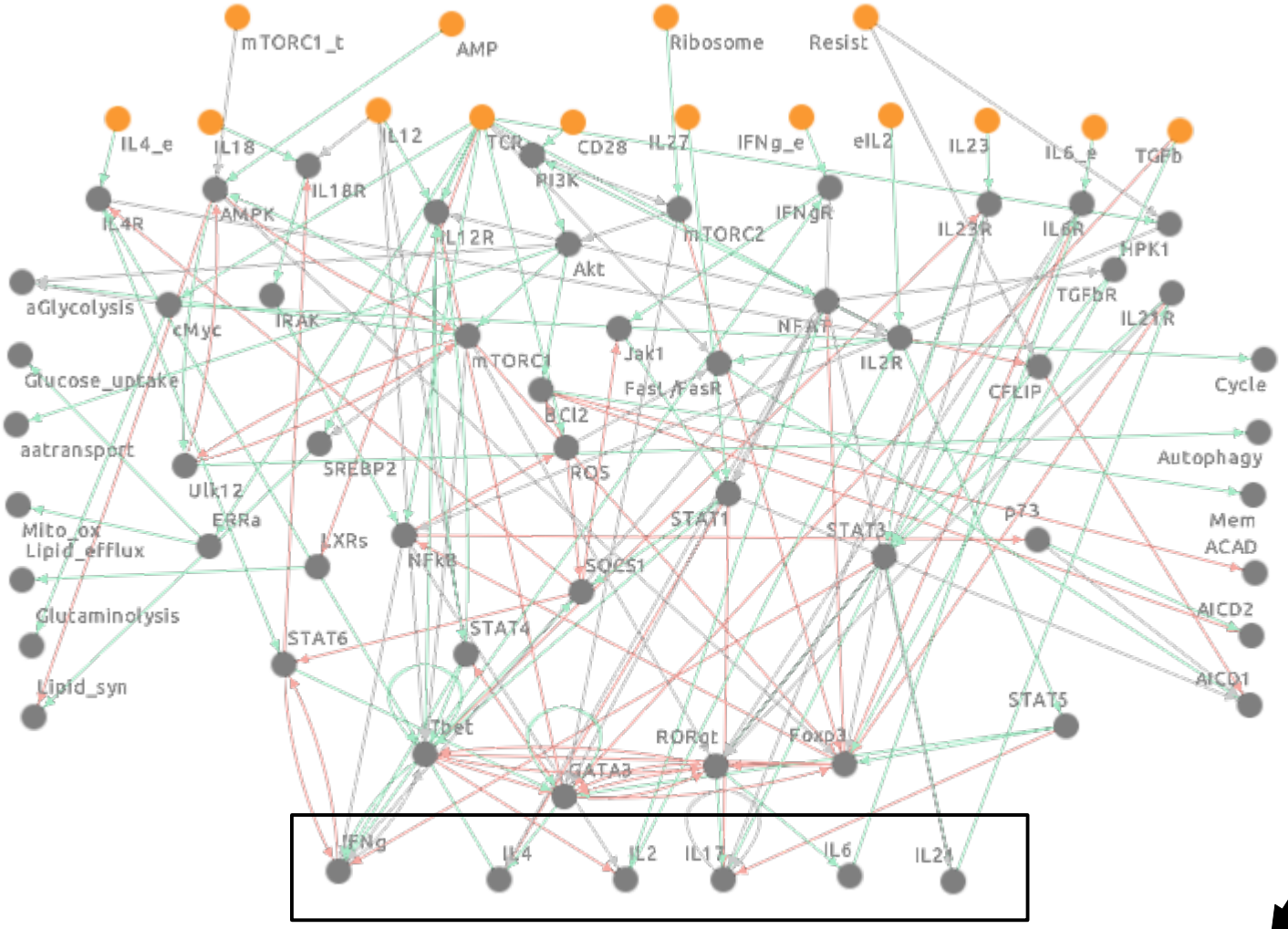
Signaling and Gene Regulation



- Internal state.
- Probabilities of apoptosis and memory formation.
- These probabilities become agent attributes.

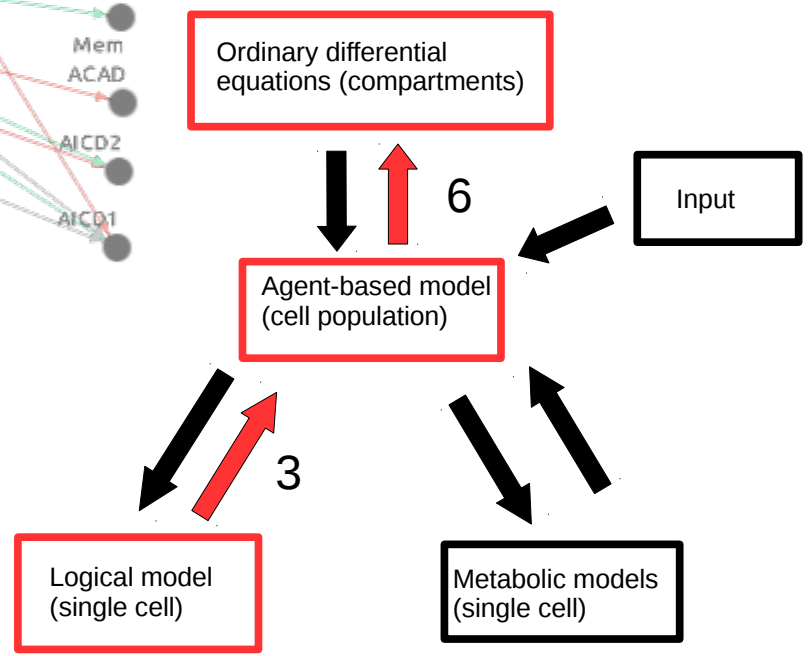


Signaling and Gene Regulation

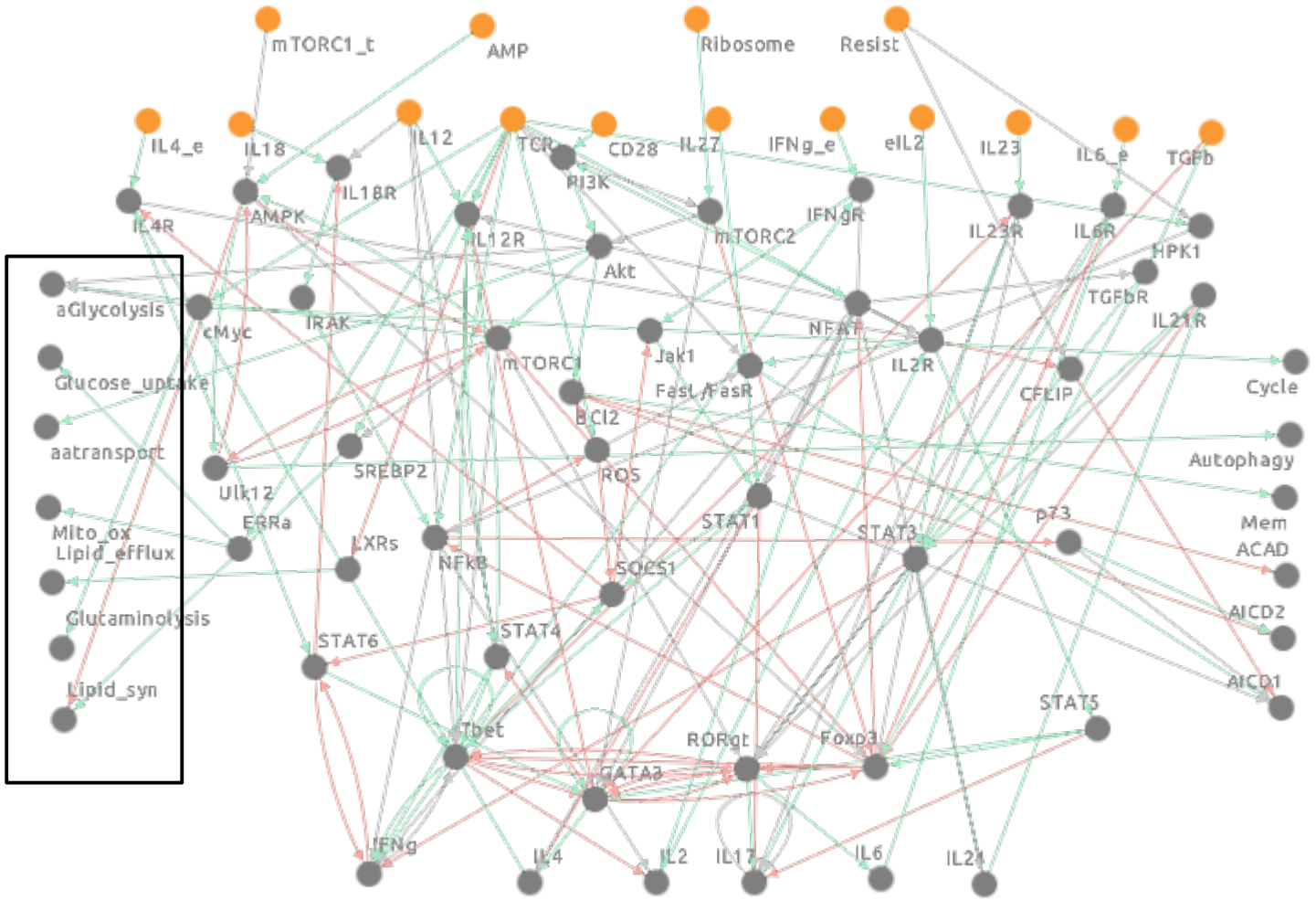


- Which cytokines can the agent produce?
- Become agent attributes used to parametrize the ordinary differential equations.

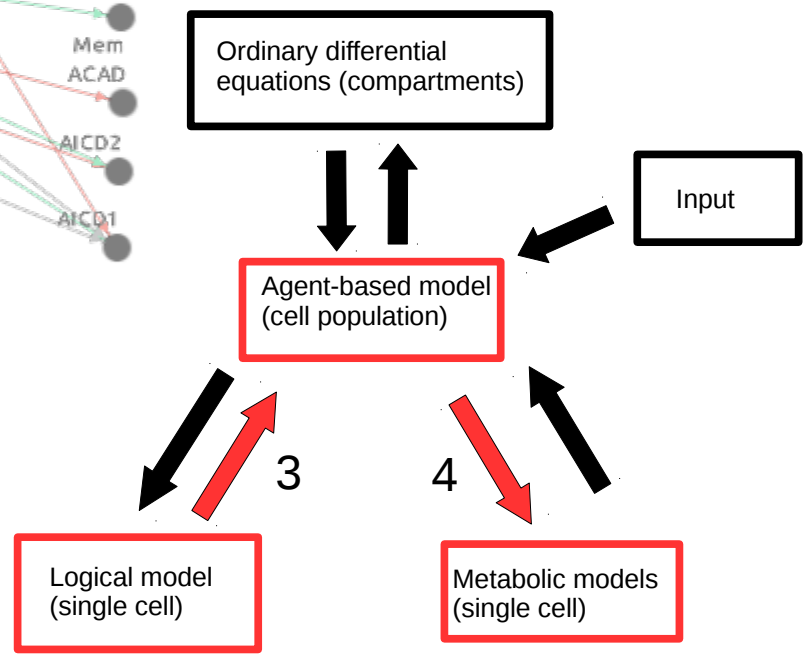
$$\frac{dC}{dt} = P - k_{deg}C + N_{in}P_{in} + N_{ex}P_{ex} + Convection$$



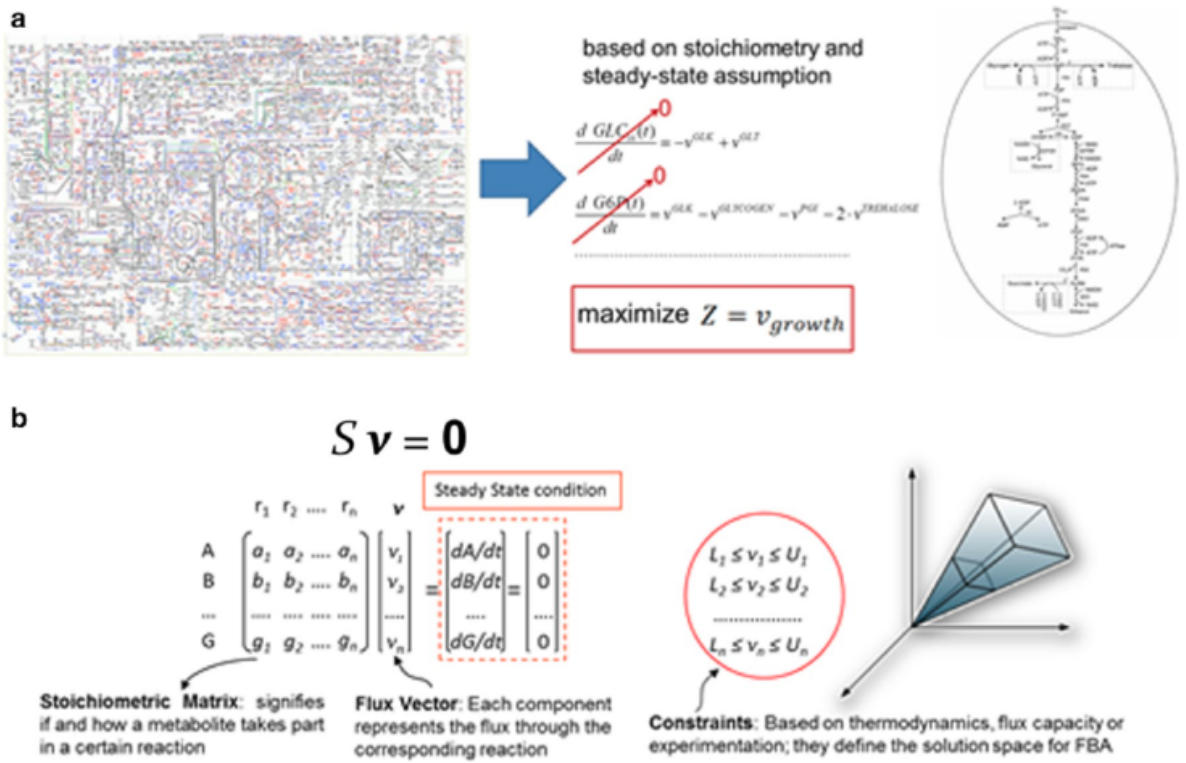
Signaling and Gene Regulation



- Metabolic events such as glucose uptake.
- Each event is a group of many metabolic reaction fluxes.
- Become agent attributes used to parametrize metabolic models.



Metabolism



Source: Simeonidis, E. and Price, N. D. (2015). Genome-scale modeling for metabolic engineering. *Journal of industrial microbiology & biotechnology*, 42(3), 327-338. https://link.springer.com/article/10.1007/s10295-014-1576-3.

- Flux balance analysis.
- Step 1: genome-scale metabolic networks.
- Step 2: mass balance of each metabolite.
- Step 3: steady-state assumption.
- Step 4: objective function (production rate of biomass or DNA).
- Step 5: constraints on the metabolic fluxes (logical model outputs).
- Step 6: linear programming.
- Step 7: optimized biomass and DNA production rates become agent attributes.

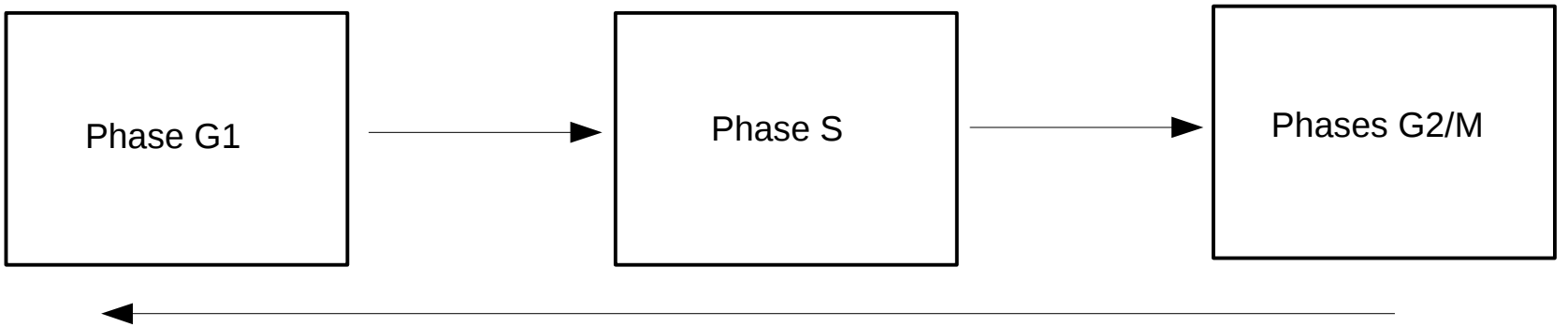
Five metabolic models, one for each phenotype.
Around 3000 metabolites and 4000 metabolic fluxes *per* model (Lal Puniya *et al.*, in preparation).

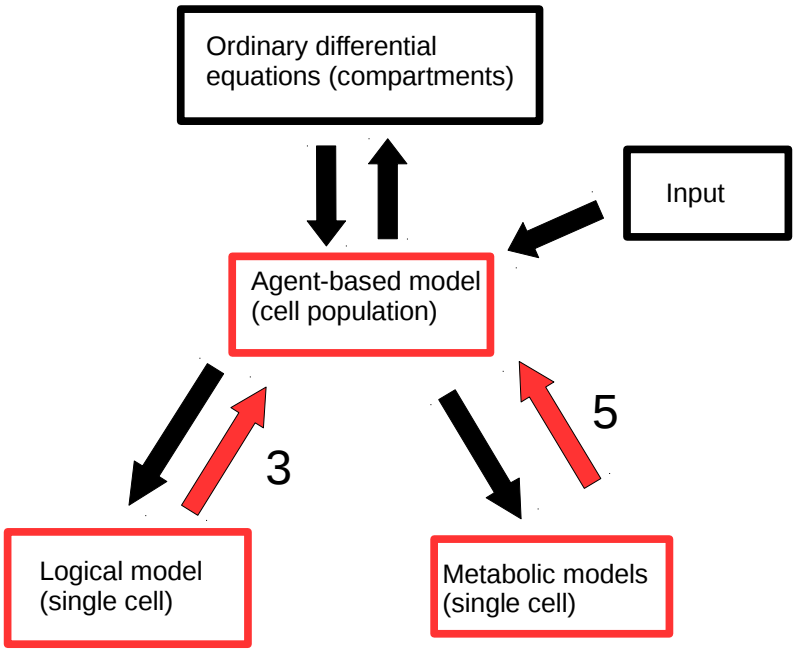
Cell Cycle and Division

Cell: cell growth.
Model: biomass production.

Cell: DNA replication.
Model: DNA production.

Cell: chromosome segregation.
Model: agent replication.

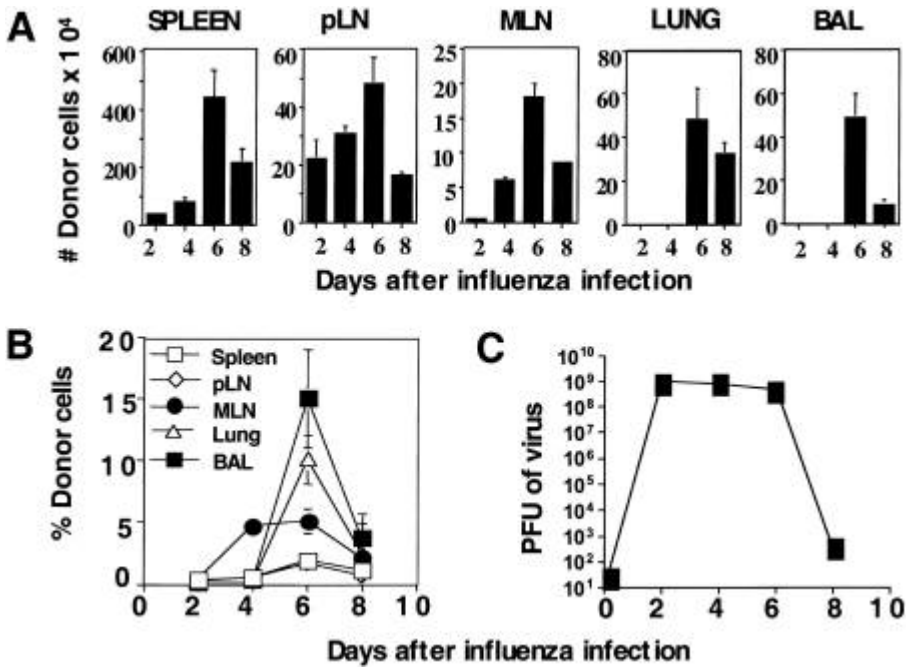




Validation

- Population dynamics in response to influenza.
- Cell differentiation in response to different cytokines.

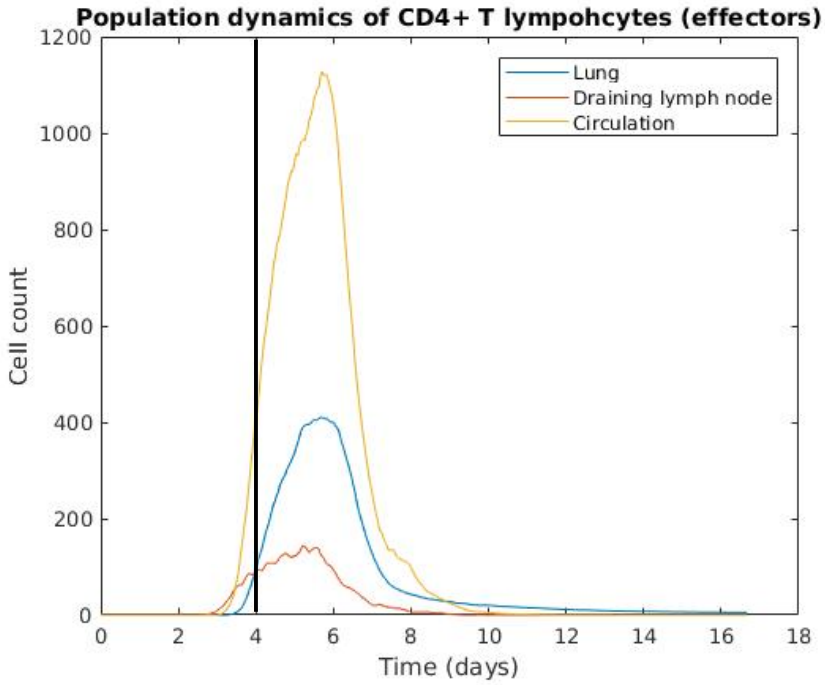
Validation: Population Dynamics

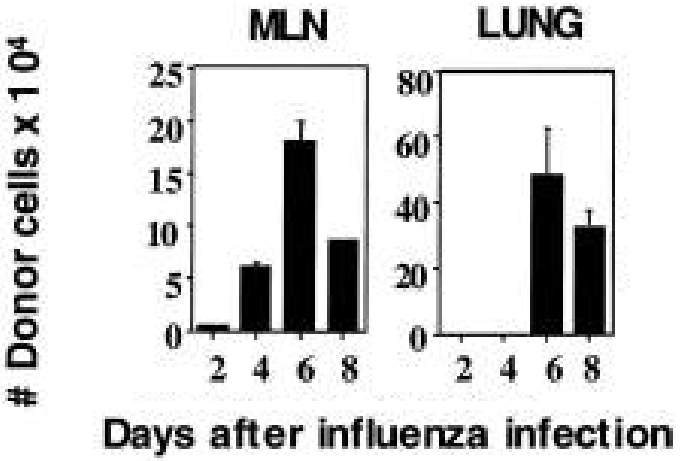


Source: Román, E., *et al.* (2002). CD4 effector T cell subsets in the response to influenza: heterogeneity, migration, and function. *Journal of Experimental Medicine*, 196(7), 957-968.

- Naive CD4+ T cells were labeled with a dye (CFSE) and transferred to mice.
- Mice were inoculated with Influenza A virus (A/PR/8/34).
- At different time points, their lungs, bronchial alveolar lavages, spleens, draining lymph nodes, and non-draining lymph nodes were sampled.
- Viral titer (viral plaque assay) and CD4+ T cell count (flow cytometry).

Validation: Population Dynamics





- 4 days post-infection.

  (a) Experiment: expansion in the draining lymph nodes (16-fold).

  (a) Simulation: expansion in the draining lymph node (nine-fold).

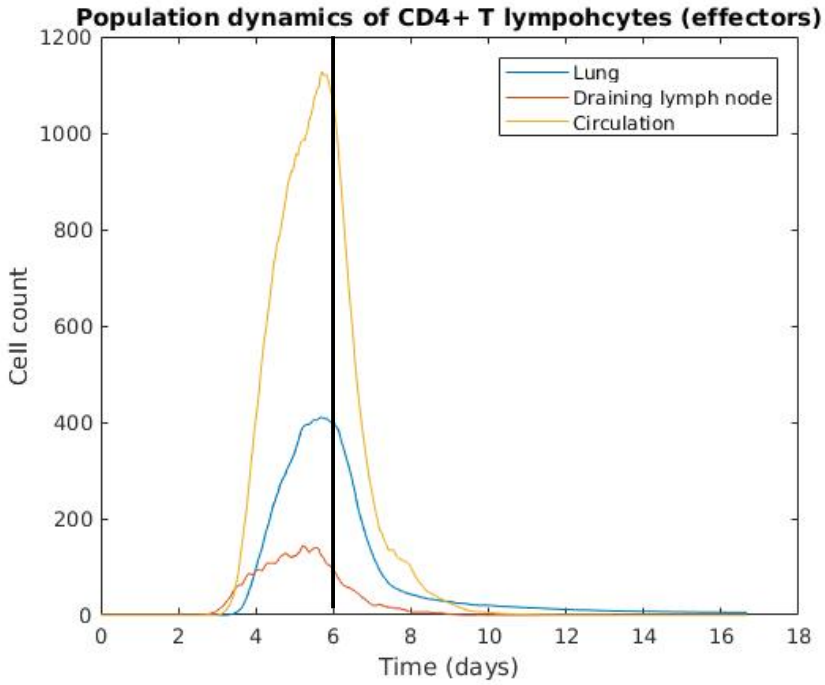
  (b) Experiment: no effector cells in the lungs (time delay between the draining lymph node and lung dynamics).

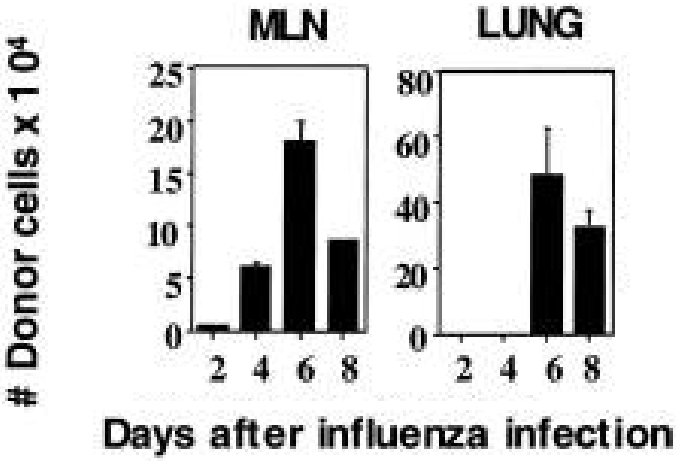
  (b) Simulation: lung dynamics are far below the peak (time delay).

- Quantitative differences: non-physical model input.
- Parametric fine-tuning.

Source: Román, E., *et al.* (2002). CD4 effector T cell subsets in the response to influenza: heterogeneity, migration, and function. *Journal of Experimental Medicine*, 196(7), 957-968.

Validation: Population Dynamics

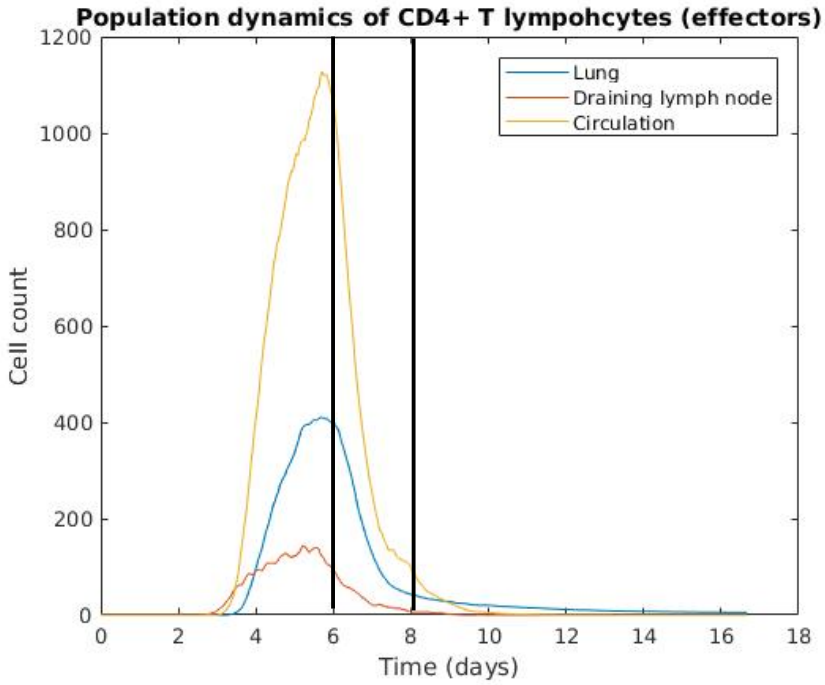


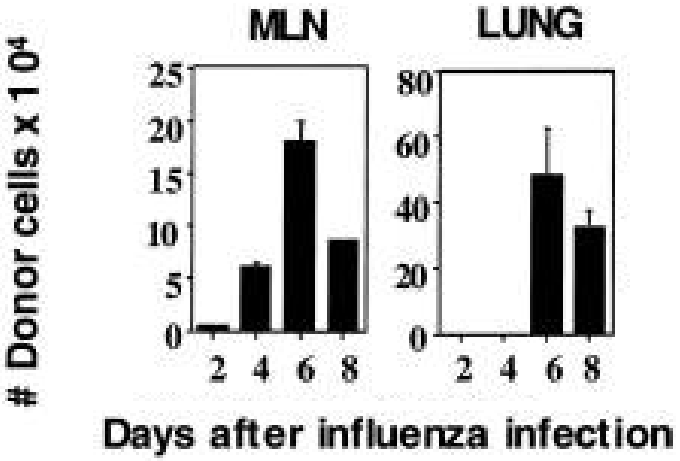


- 6 days post-infection.
- Experiment: the response peaked in all sampled tissues.
- Simulation: validated.

Source: Román, E., *et al.* (2002). CD4 effector T cell subsets in the response to influenza: heterogeneity, migration, and function. *Journal of Experimental Medicine*, 196(7), 957-968.

Validation: Population Dynamics

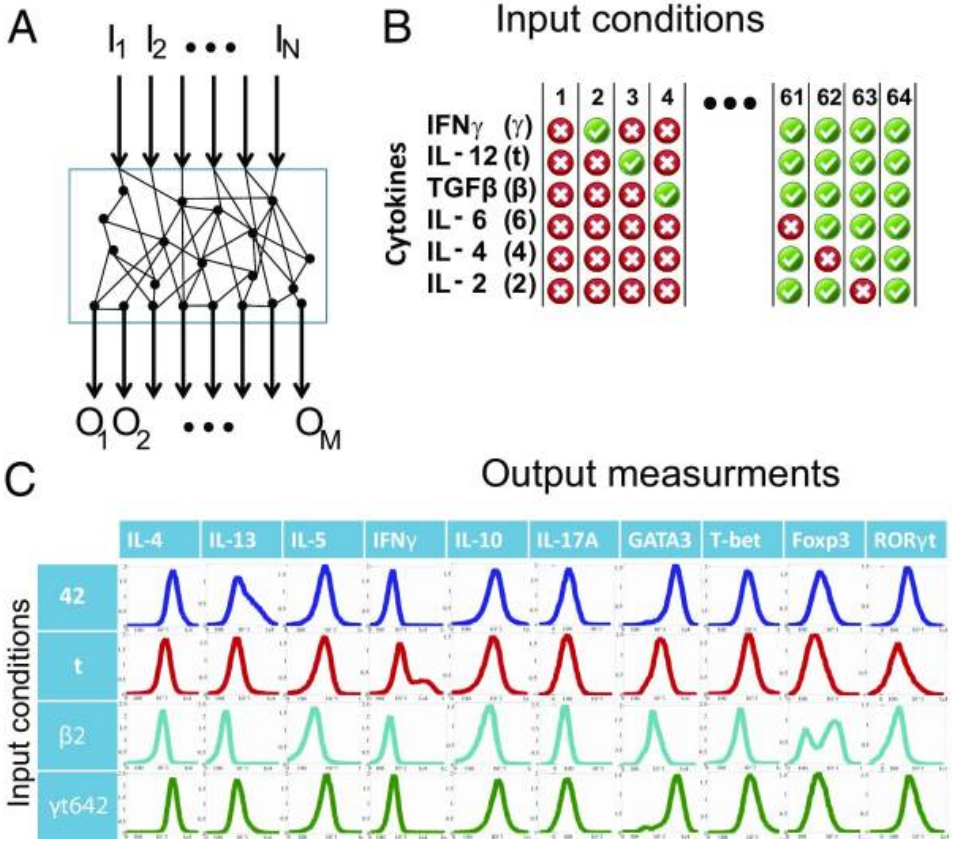




- 8 days post-infection.
- Experiment: between day 6 and day 8, the populations were declining.
- Simulation: declining dynamics validated.

Source: Román, E., *et al.* (2002). CD4 effector T cell subsets in the response to influenza: heterogeneity, migration, and function. *Journal of Experimental Medicine*, 196(7), 957-968.

Validation: Cell Differentiation



Source: Eizenberg-Magar, I., *et al.* (2017). Diverse continuum of CD4+ T-cell states is determined by hierarchical additive integration of cytokine signals. *Proceedings of the National Academy of Sciences*, 201615590.

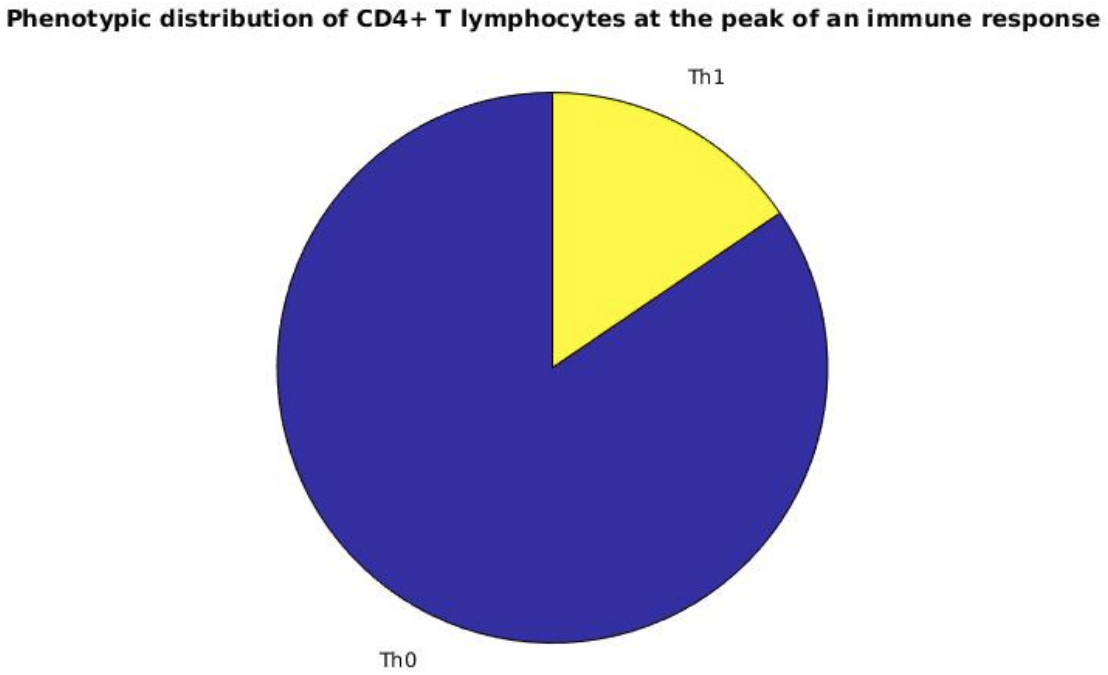
- Naive CD4+ T cells from mouse spleens.
- Cultured with TCR, CD3, and CD28 signals.
- Supplemented with cytokines: 64 combinations of IL2, IL4, IL6, IL12, IFNg, and TGFb.
- For example, IL2 is either present at 5 ng/mL or absent.
- Expression levels of four transcription factors and six cytokines (intracellular staining and flow cytometry).
- Classification into phenotypes.

Validation: Cell Differentiation

Cytokine Combination	Phenotype
IL12	Th1
IL2 and IL4	Th2
TGFb and IL6	Th17
TGFb and IL2	Treg

- Same input from the influenza experiment.
- No cytokine production due to the input.
- Reproduced four experimental cytokine combinations in the three compartments.

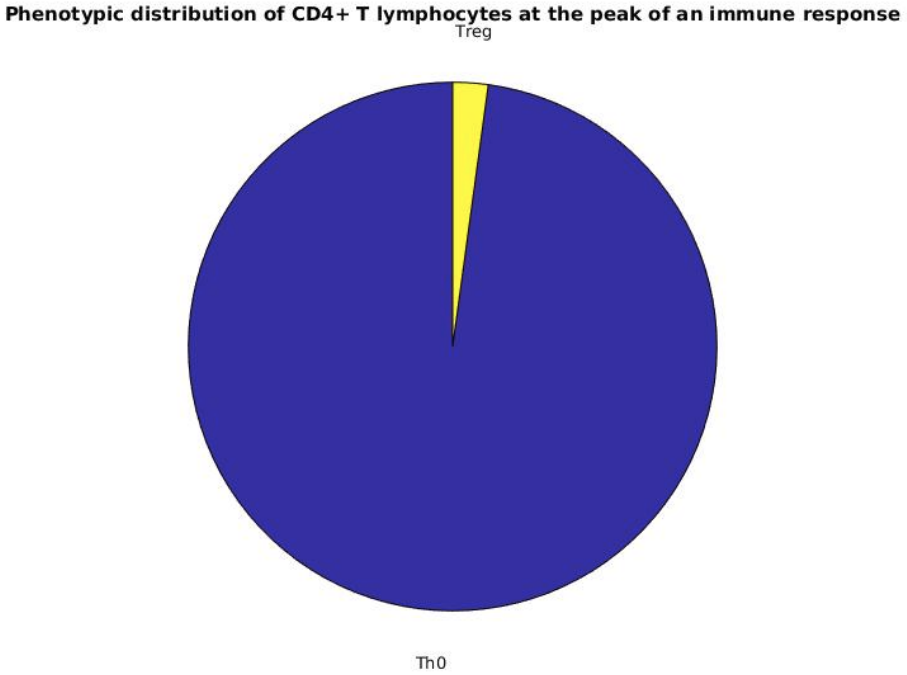
Validation: Cell Differentiation

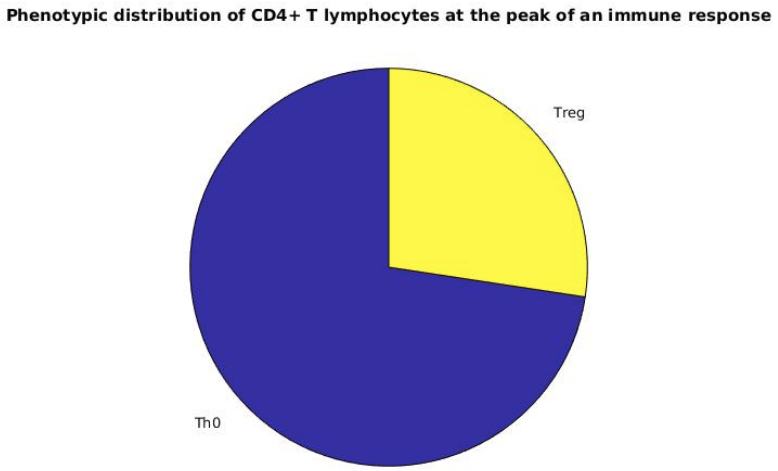


- Phenotypic distribution in the effector cell population at the peak of the immune response in the presence of IL12.

Cytokine Combination	Phenotype
IL12	**Th1**
IL2 and IL4	Th2
TGFb and IL6	Th17
TGFb and IL2	Treg

Validation: Cell Differentiation





- Model is differentially sensitive to different cytokines.
- Top: experimental dosage of TGFb and IL2; Treg response is weaker than the Th1 counterpart.
- Bottom: 10 times the experimental dosage.
- Higher cytokine concentrations, stronger response.

Cytokine Combination	Phenotype
IL12	Th1
IL2 and IL4	Th2
TGFb and IL6	Th17
TGFb and IL2	**Treg**

Further Validation Studies

- Differentiation into more complex phenotypes.
- Effects of IL2, IL4, and IFNg on metabolism.
- Effects of chronic inflammation.

Conclusions

- Multi-scale model of CD4+ T lymphocytes.
- Four modeling frameworks: ordinary differential equations, agent-based model, logical model, and metabolic models.
- Three numerical methods: finite difference method, Monte Carlo method, and linear programming.
- Proof of concept.

Future Work

- Model other immune cell types and their relevant cytokines and antibodies.
- Direct interactions between the modeled immune cell types.
- Virtual immune system: a multi-scale platform for immunologists.
- For more information: kwertheim2@unl.edu (Dr Kenneth Y. Wertheim), thelikar2@unl.edu (Dr Tomas Helikar), http://helikarlab.org/ (lab website).