INTRODUCTION
• During infection, T Helper (Th) cells choose which immune weapons to deploy based on the type of invading parasite. This process is called effector choice.
• During effector choice, Th cells produce molecular messages called cytokines.
• Th1 cells fight intracellular parasites (e.g. viruses) and produce IFN-γ.
• Th2 cells fight extracellular parasites (e.g. worms) and produce IL-4.
• Previous work predicts that Th cells use quorum sensing during effector choice.
• Quorum sensing = change in cellular behavior as cell density changes.
• Quorum sensing among Th cells could manifest as an impact of cell density on effector choice, especially if this impact is enabled by cytokine signaling.

EXPERIMENTAL METHODS
• Th cells expressing fluorescent IFN-γ were extracted from transgenic mice to visualize Th1 differentiation (IACUC Protocol Number 2110-17).
• Th cells were sorted into different chambers of a coverslip, in which different combinations of cytokine signaling were permitted.
• Th cells assorted into thousands of replicated microwells.
• Th cells were stimulated for survival and Th1 differentiation.
• The coverslip was imaged every 2 hours for 48 hours to measure cellular positioning, viability, and Th1 differentiation for every microwell.

STATISTICAL METHODS
• Bayesian multilevel modeling and subsequent model averaging quantified how Th1 differentiation in each microwell depended on:
  - Timepoint,
  - Type(s) of permitted cytokine signaling,
  - Average distance between cells (a proxy for cell density),
  - Number of cells (a second proxy for cell density),
  - Interactions of the above predictors.

RESULTS: MAIN EXPERIMENT
• Evidence for quorum sensing: Th1 differentiation rate increases with cell density
• Evidence against quorum sensing: cytokine signals do not affect differentiation

RESULTS: FOLLOWUP EXPERIMENT
• Cytokine signals now strongly control Th1 differentiation.
  • The increase in Th1 differentiation with cell density is still observed.

DISCUSSION
• Cytokine signaling and cell density both modulate Th1 differentiation, though the interaction between these two factors was not directly observed.
• The evidence for quorum sensing among Th cells is compelling, but partial.
• Collectivity among immune cells, such as quorum sensing, indicates that effective immunotherapies may have to modulate the dynamics of cellular interactions.

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