Integration of immune components into NAMs:

Scientific essentials towards qualification

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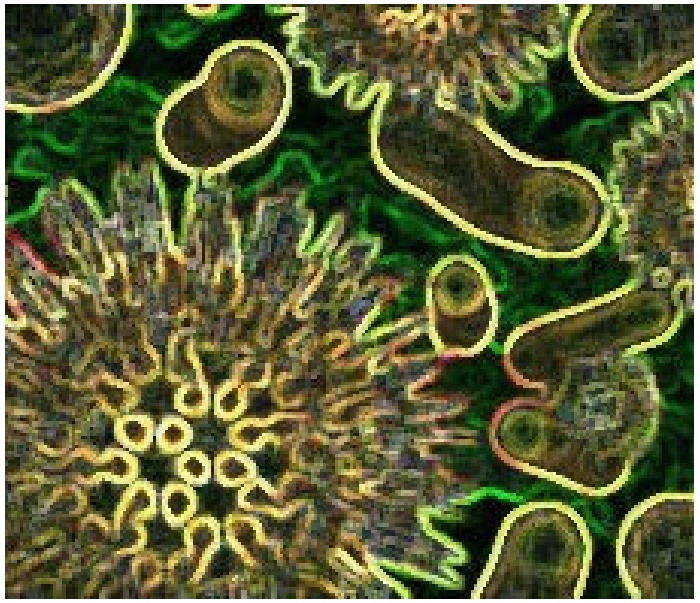
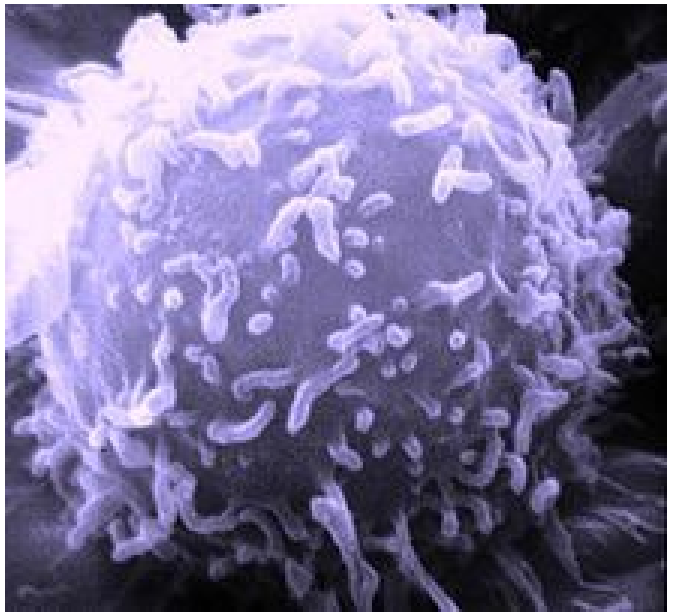
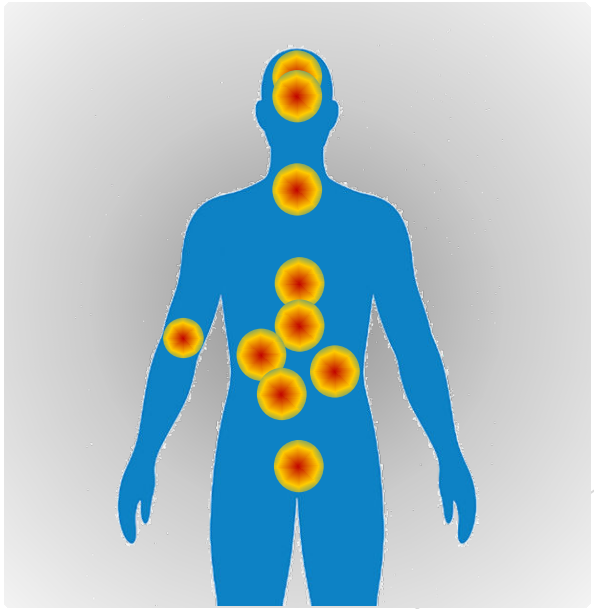


infection



cancer

autoimmunity



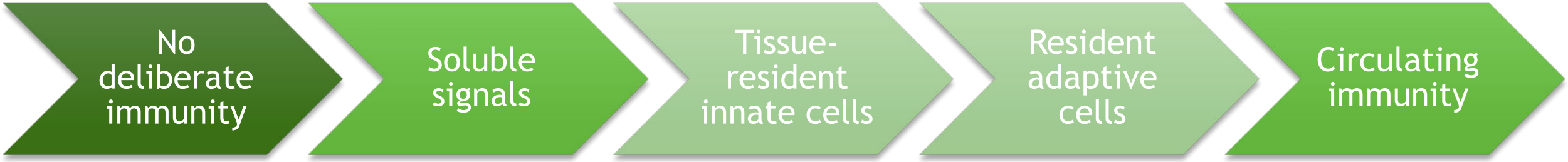
chronic inflammation

Today's discussion:
Integration of immune components into NAMs

- What specific immune functions matter?
- What biological constraints must inform immune-competent NAMs design?
- What evidence would make these systems credible for use and qualification?

What Does 'Immune-Competent' Mean?

Levels of complexity



- Cytokines
- Lipopolysaccharide
- Complement

- Macrophages
- Dendritic cells
- Neutrophils
- Mast cells
- Microglia (brain)
- Kupffer cells (liver)

- Resident T cells
- Resident B cells
- Resident dendritic cells

- PBMCs
- Monocytes
- Natural killer cells
- T cells, B cells

"Every cell is an immune cell"

- Endothelium
- Fibroblasts

What Does 'Immune-Competent' Mean? What must the model do?

1. What functions might an immune-competent NAM actually perform?
   - Innate or adaptive? Inflammation or antigen-specific? Acute or long-term responses?
   - Stimulus? Measurable outputs?

2. Which of these functions are essential for drug safety or efficacy testing? In what context(s)?

- How do we keep systems tractable?
- Relevance to your research or workflow?

Notes

- Need to know mechanism to know what you want to model
  - For regulatory purposes… drug dev… go with minimal model
  - For early stage mechanistic experiments… you may need a more complex model, or multiple models
- To decide whether immunity is needed:
  - Is it the target of the therapy? Or, essential to the mechanism of action? Or, mech of toxicity?
  - Or, if the immune cell fundamentally alters the function of the other cells in the model (e.g. microglia)

Biological Design Considerations and Constraints

- Cell sourcing
- Migration & dynamics
- Population variation

Cell Sourcing

- Consider immune cell sources:
  - Primary human cells
  - Cell lines
  - Cell lines with genetic modifications (reporters, knock outs)
  - iPSC-derived cells

1. Brainstorm specific examples
2. Strengths and limitations for each

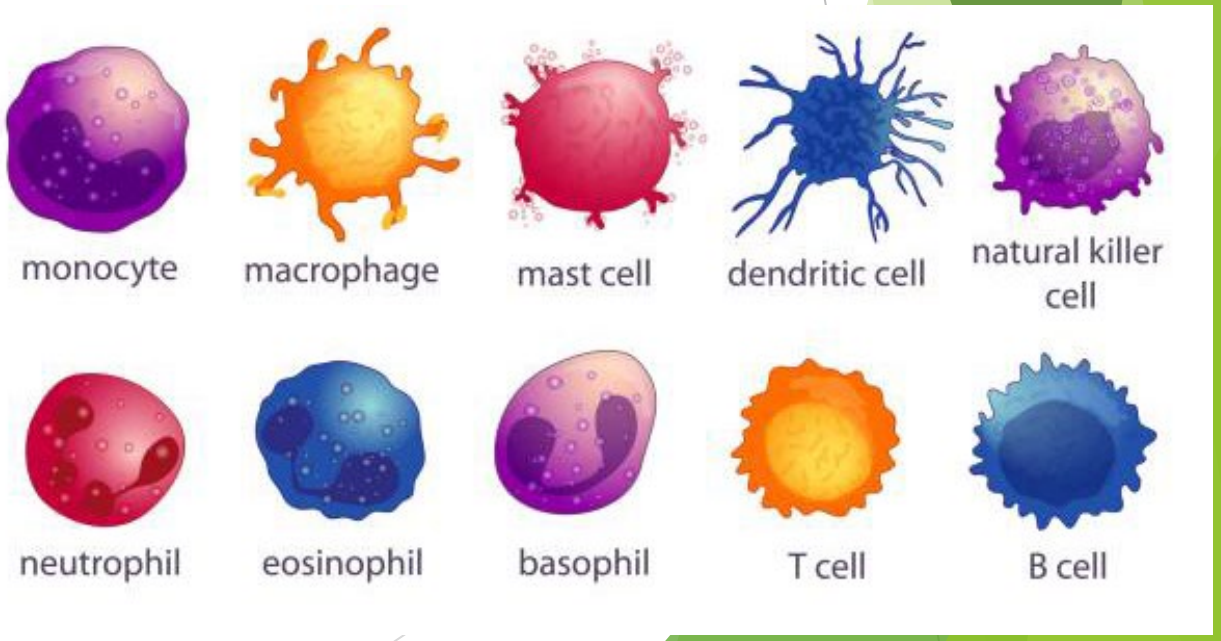


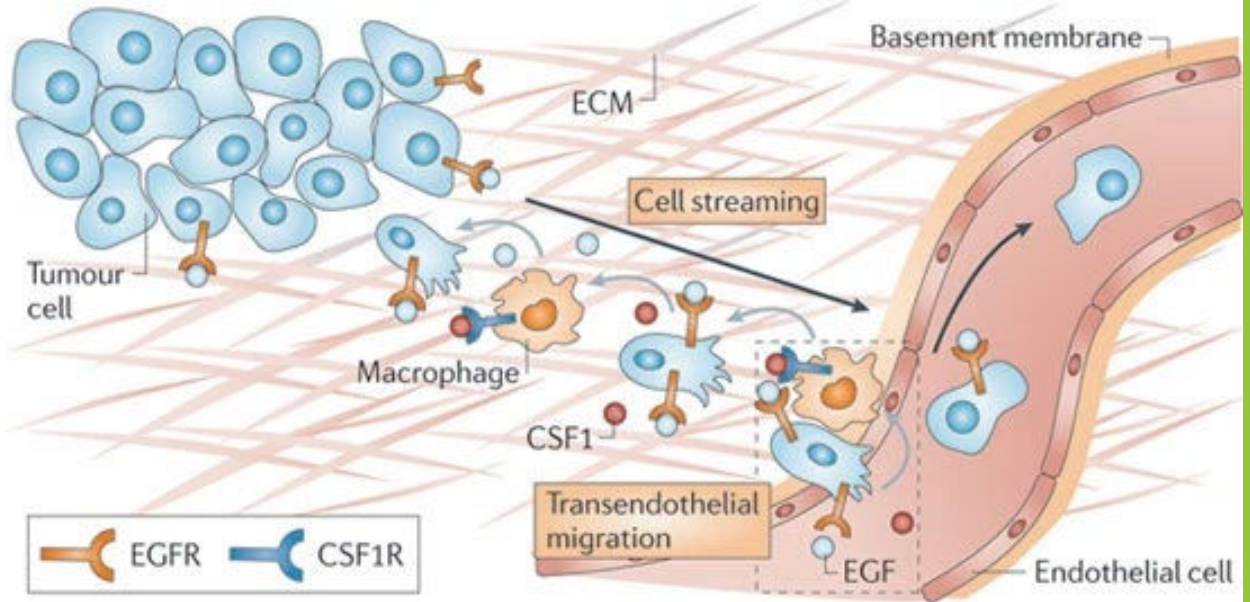
Image Credit: Penn State, Stem Cell & Regen Biol Program

	Sources/Examples	Strengths	Limitations
Primary human cells			
Cell lines			
Modified cell lines			
iPSC-derived cells			

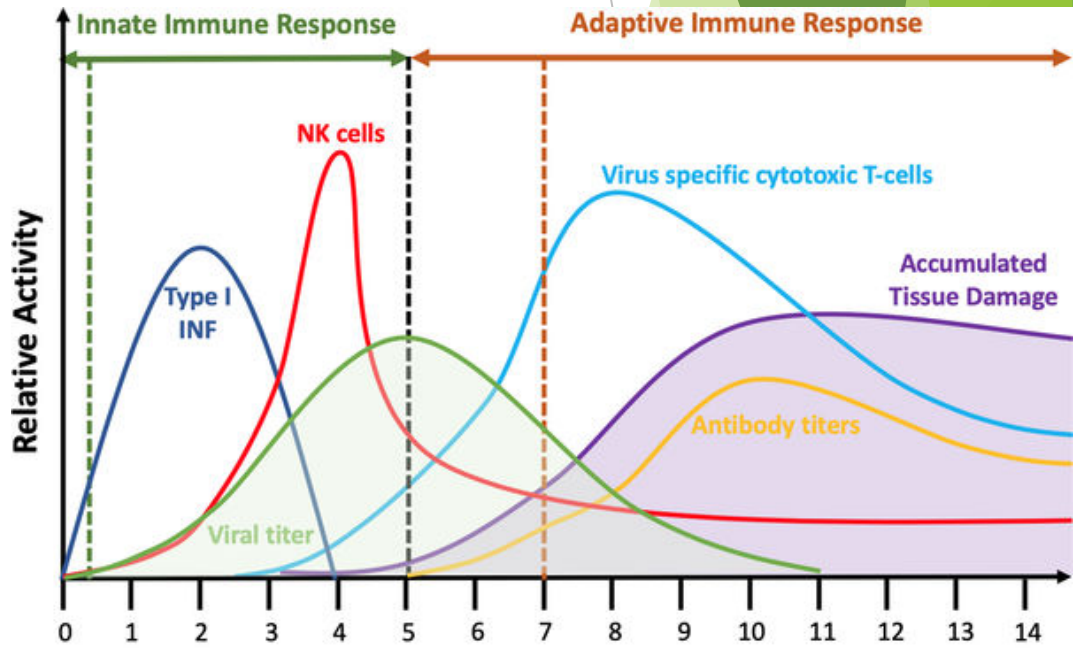
Migration & Dynamics

1. Lymphocytes are **migratory.** Incorporate migration, chemotaxis, invasion?
   - Within one organ
   - Between organs … trafficking? recirculation?
   - Vasculature, chemotaxis

2. Responses vary in **timescale**, min – wk. Impact on NAMs design for decision making?

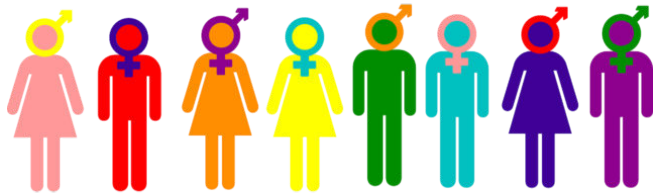


Roussos et al, *Nat Rev Cancer,* 2011



Sego et al, *PLOS Comp Biol*, 2020

Population variation



- What variables likely impact immune function?
  - Demographic variables?
  - Individual experience/exposures?
- To what extent have these been included in NAMs so far?
- When could omissions or inclusion mislead decision-making?
  - Notes
    - Heterogeneity (more variables) adds cost to tests, needs clinical-trial-style multi-variate statistics
    - Missing heterogeneity may miss idiosyncratic responses

- Sex differences
- Age – variations in aging quality
- HLA diversity
- Tissue imprinting
- Trained immunity
- Diet/microbiome exposure
- Chronic inflammatory baselines
- Prior drug exposure
- Ancestry
- Race

More notes

- Need input from each perspective – developer, pharma, reg, etc
- HLA – Japanese estimate of how many iPSC lines would be needed to be representative in that country's HLA

Towards Utility for Decision Making

What would make immune-competent NAMs credible for decision making?

- What evidence would you find convincing to support adoption an immune-competent NAM for predictions?
  - Composition definition
  - Validation strategy
  - Reproducibility

- Benchmark compounds?
- Reproducing known immune toxicities?
- Cross-lab reproducibility?
- Defined performance metrics?
- Reference immune challenges (e.g., LPS, checkpoint inhibitors)?

Notes on prepping for decision making utility

- **Need QC on cells, how stored, endotoxin free reagents**
- **Validation**
  *** concerns about discordance b/w animal vs NAM**
  *** consider dosage on-chip vs in patient**

Final thoughts

- Centrality of immune function in drug development
- Need to define function of interest for each model
- Challenges/Opportunities:
  - Cell sourcing
  - Migratory & dynamic qualities
  - Population variation
  - Reference conditions/benchmarking
  - Standardization of outputs/readouts