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New technologies for studying human immunity

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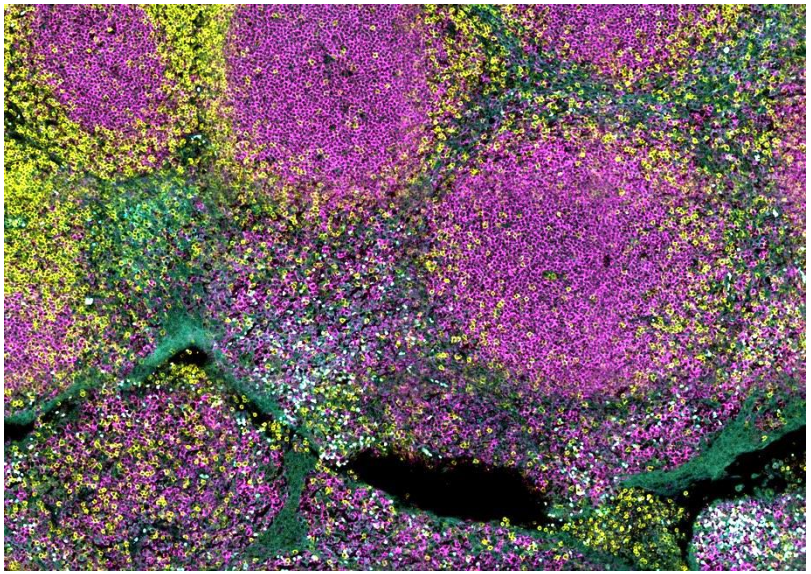


New strategies: Human immunology is ideal for a systems approach

- We have learned a great deal from mouse models, but they have been only sporadically successful in understanding human diseases
- We need to **study the human immune system directly**
- While we can't do many of the same kinds of experiments, **new technologies** present a number of good options

- Cell types and cytokines can be measured in blood and tissue samples using **high throughput** techniques
- Many perturbations of the system (vaccines, infectious diseases, cancer, drug treatments, environment) involve the immune system

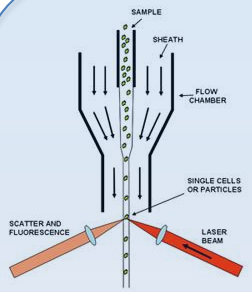
The Stanford Human Immune Monitoring Center: Assisting all stages of translational immunology

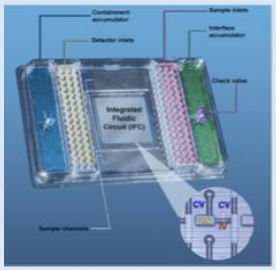
Trial design

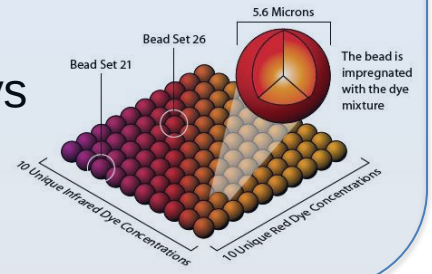
Processing and banking

New Technology

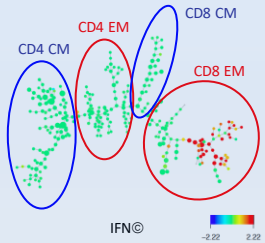
Assays

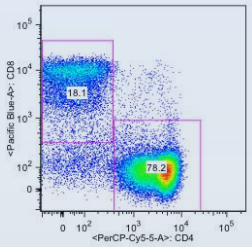






Analysis





Data Mining

Dimensions		Gender: All Genders	
		Measures	
Assay-Analyte	Assay	Mean	N
All Assays	Flow phenotyping	42.3	5,800
	Luminex/MSD	2,399.4	48,505
	Phospho Flow	443.0	3,024

Our Motto: Immunology for the People!

Good news: we have technologies for studying correlates of protection

Mass cytometry

→Measuring immune parameters at single cell level

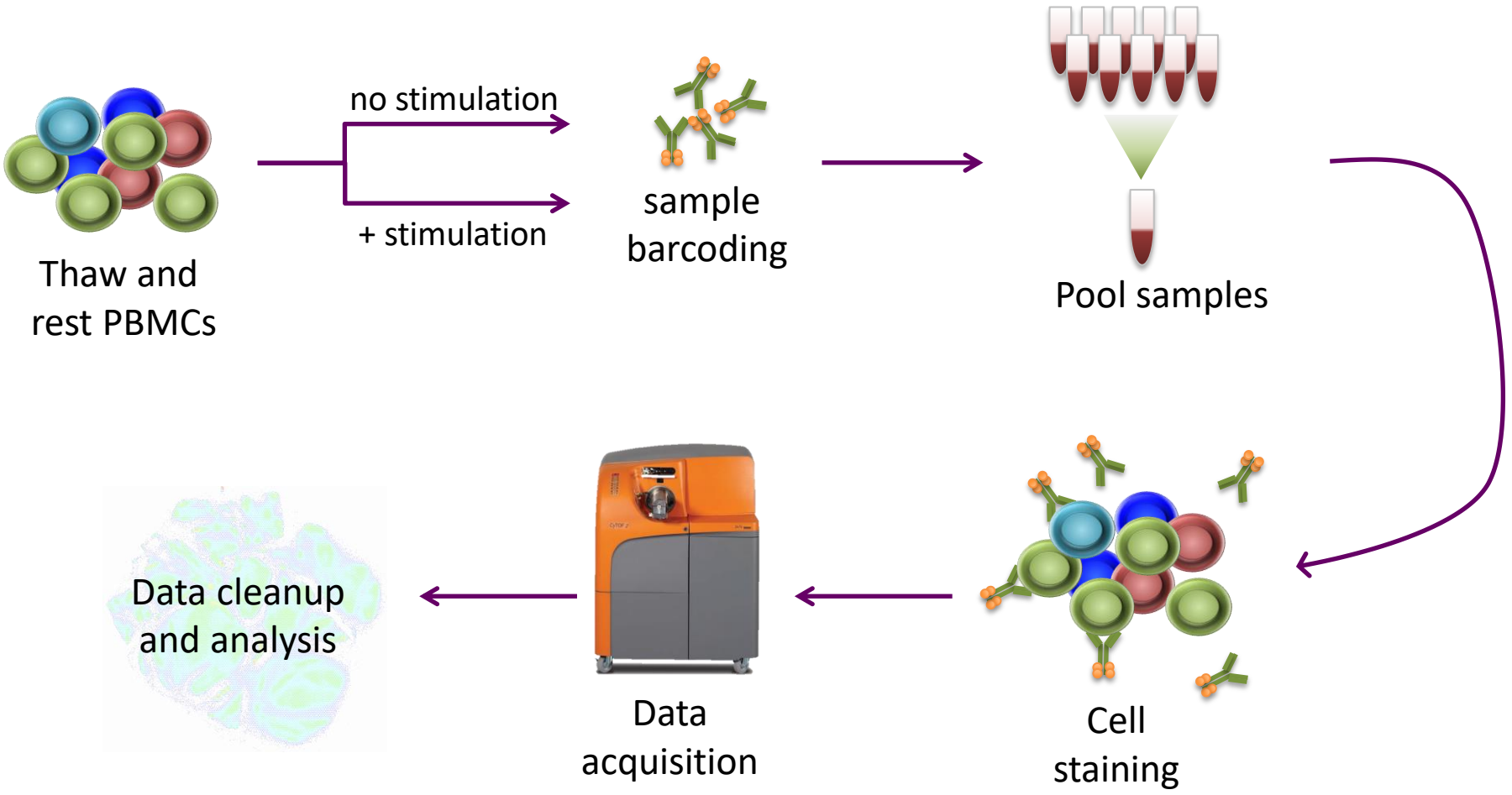
Single cell TCR sequencing & ligand discovery

→Identifying relevant T cell targets

Immune organoid models

→High throughput vaccine/adjuvant testing platform

Mass cytometry to analyze phenotype and function of PBMC samples



Measures 30-50 markers all at once

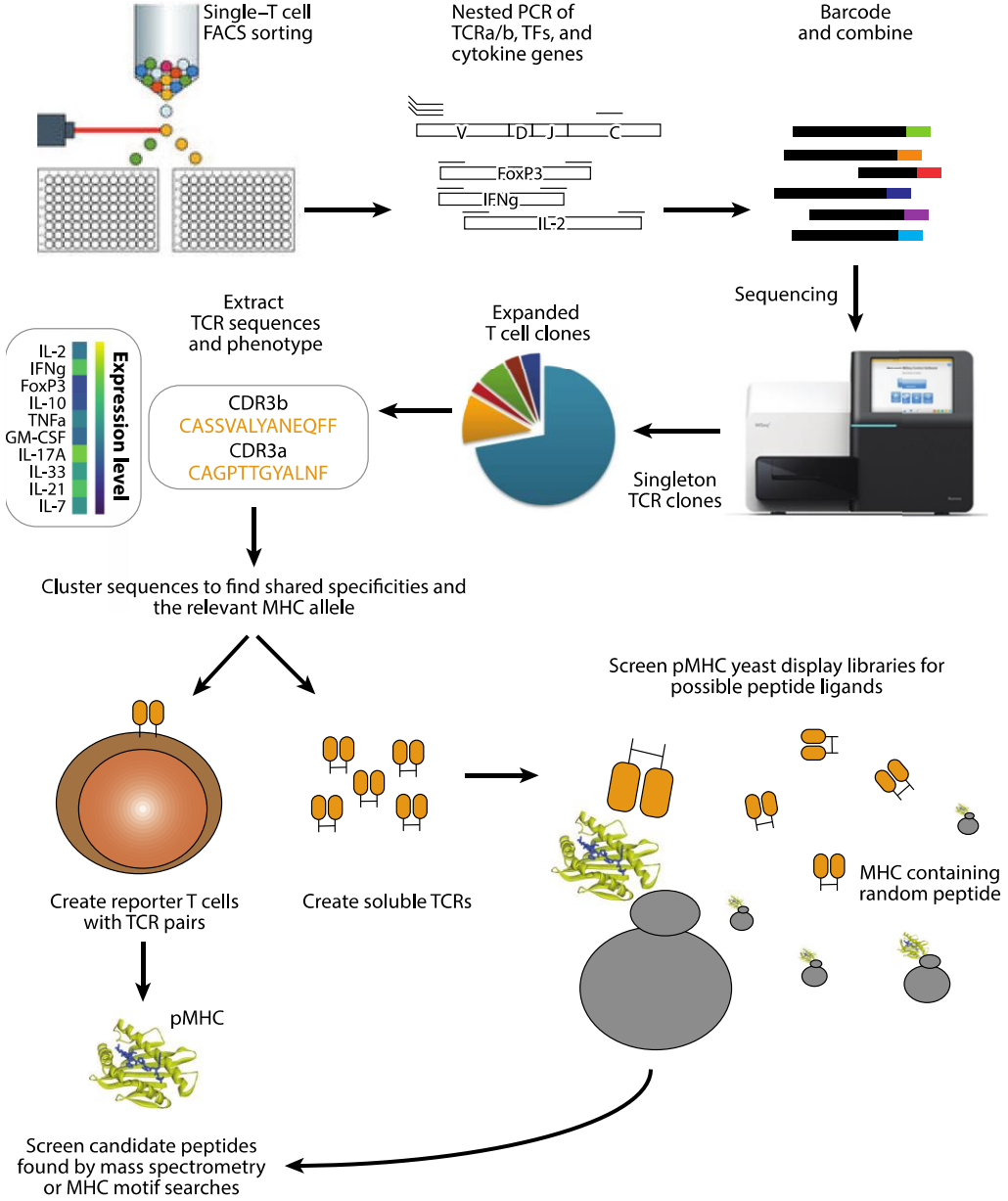
-Complex combinations of expression that may be missed otherwise

-No need to focus on only one cell type or split samples for multiple marker panels

CyTOF: potential for identifying correlates of protection

- Single cell resolution of high dimensional data has provided us with a picture of what cellular changes occur with immune perturbations, age, environment
- Pre- and post-vaccination samples can be compared and analyzed at the individual and group levels
  - Managing human immune variation
- Old (flow-style manual gating) and new (clustering algorithms) analysis tools are available
- Not ideal for detecting very small changes/cell populations

Tools for T cells: a rapid pipeline for going from T cells in any disease to understanding their function and specificity



-T cell responses are often overlooked during vaccine protection studies.

-Each person's T cell repertoire is unique; finding identical sequences isn't sufficient.

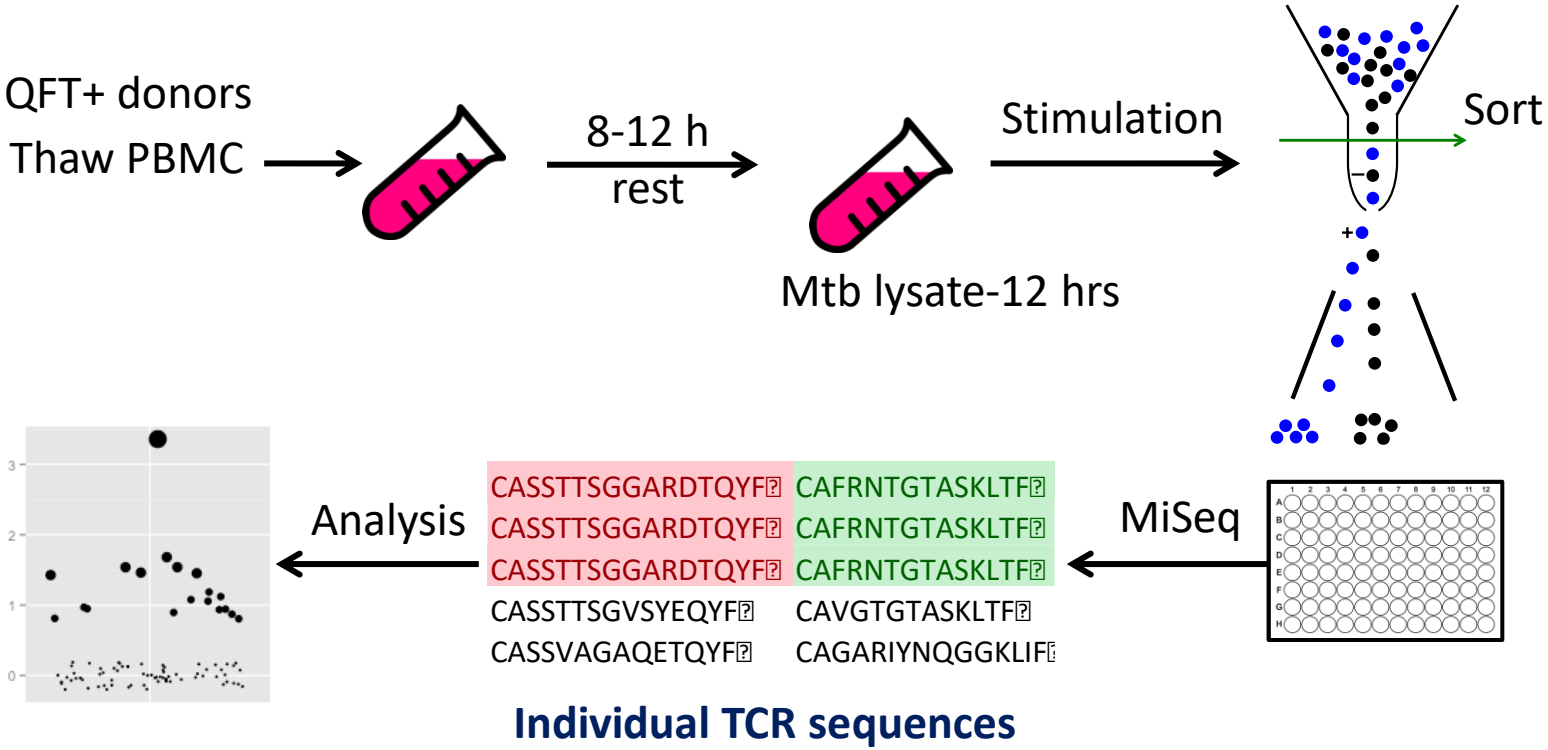
Han et al. Nat Biotech 2014
Glanville, Huang et al., Nature 2017
Davis et al Nat. Immunol. 2017

GLIPH: Grouping of Lymphocyte Interactions by Paratope Hotspots (Jacob Glanville)

- Groups TCR sequences that likely recognize the same peptide-MHC ligands (**convergence groups**)
- Find dominant **motifs** shared by individuals
- Estimate the **diversity** of a T cell response

- Potential to analyze $\alpha\beta$ T cell responses directly from sequence

A collection of 5700 Mtb specific CD4+TCRs (Huang Huang)

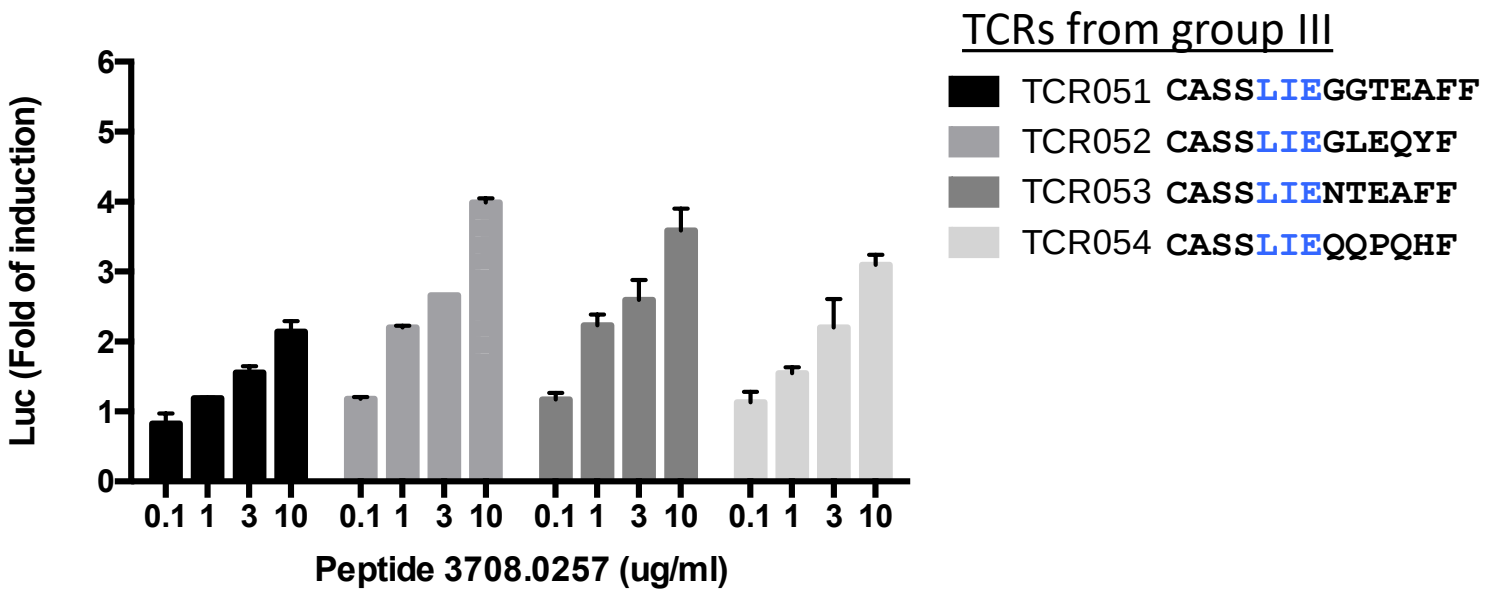


Individual TCR sequences

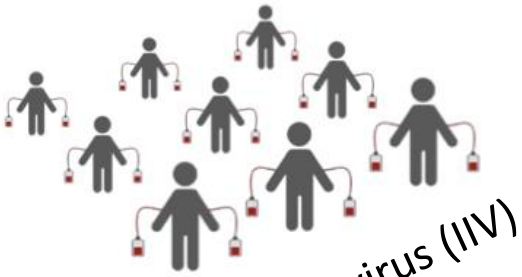
Single T cell seq method from Han et al. Nat Biotech 2014

Glanville, Huang et al., Nature 2017

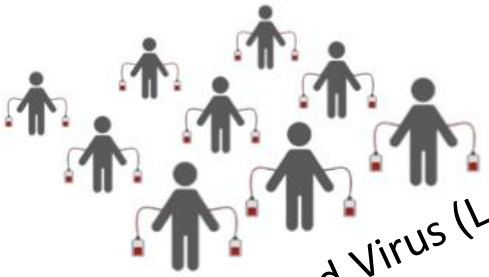
T cell receptors from an identified convergence group recognize the same Tb peptide



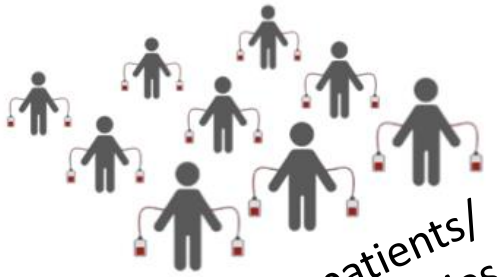
Assembling the influenza TCR-ome



Killed virus (IIV)



Attenuated Virus (LAIV)



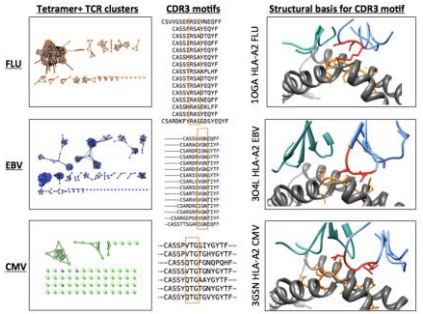
Infected patients/
Challenge studies

Collect
Clinical response
Vaccine metrics
Immune metrics

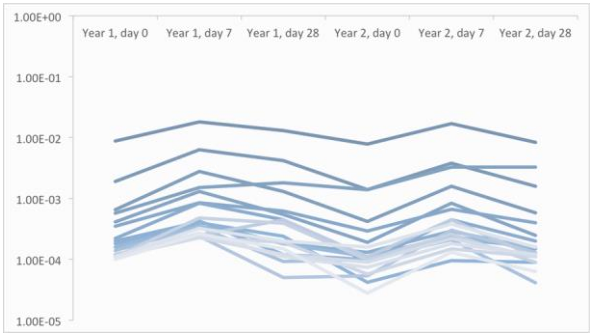
Sequencing
HLA typing
TCR sequencing
TCR Single cell

Compute
TCR specificity groups
HLA concordance

Influenza-TCR-ome



Expanded TCR sequences
Over two years of flu vaccination



Infection status
Probable HLA types
CD4 and CD8 epitope responses
Correlates of protection
100s of other antigens…

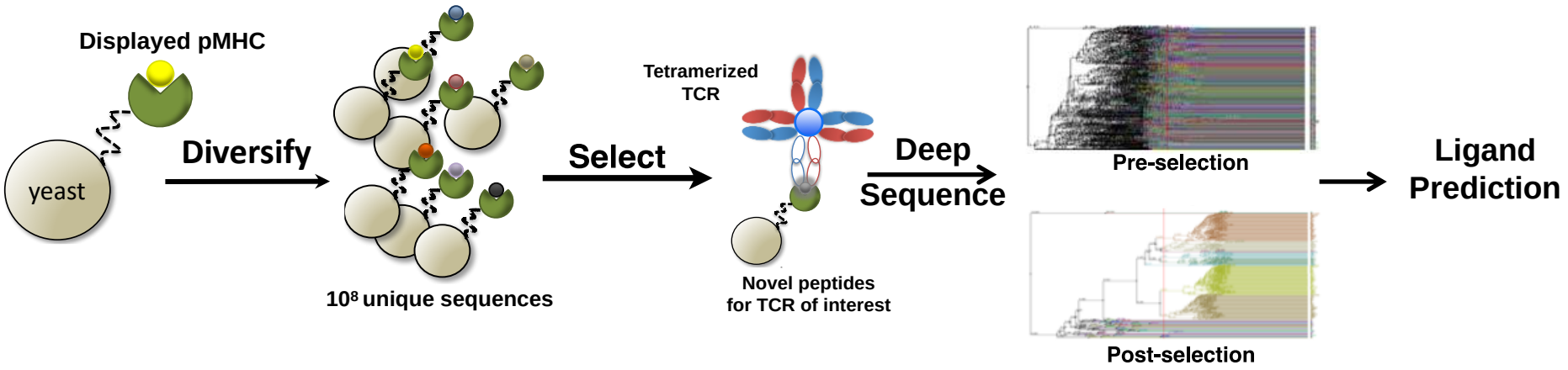
Searching for T cell targets is straight-forward when there are a small number (<500) of candidate peptides.

What do we do when the pool of possible T cell targets is large (complex pathogens) or unknown?

Ligand Discovery: going from TCRs of interest to antigen

Classical method: make individual reporter T cell lines and survey

New method: yeast display (Birnbaum et al., Cell 2014)
→enriches pMHC binders from a yeast library



Chris Garcia, Michael Birnbaum, Marvin Gee

In vitro models of the adaptive immune response

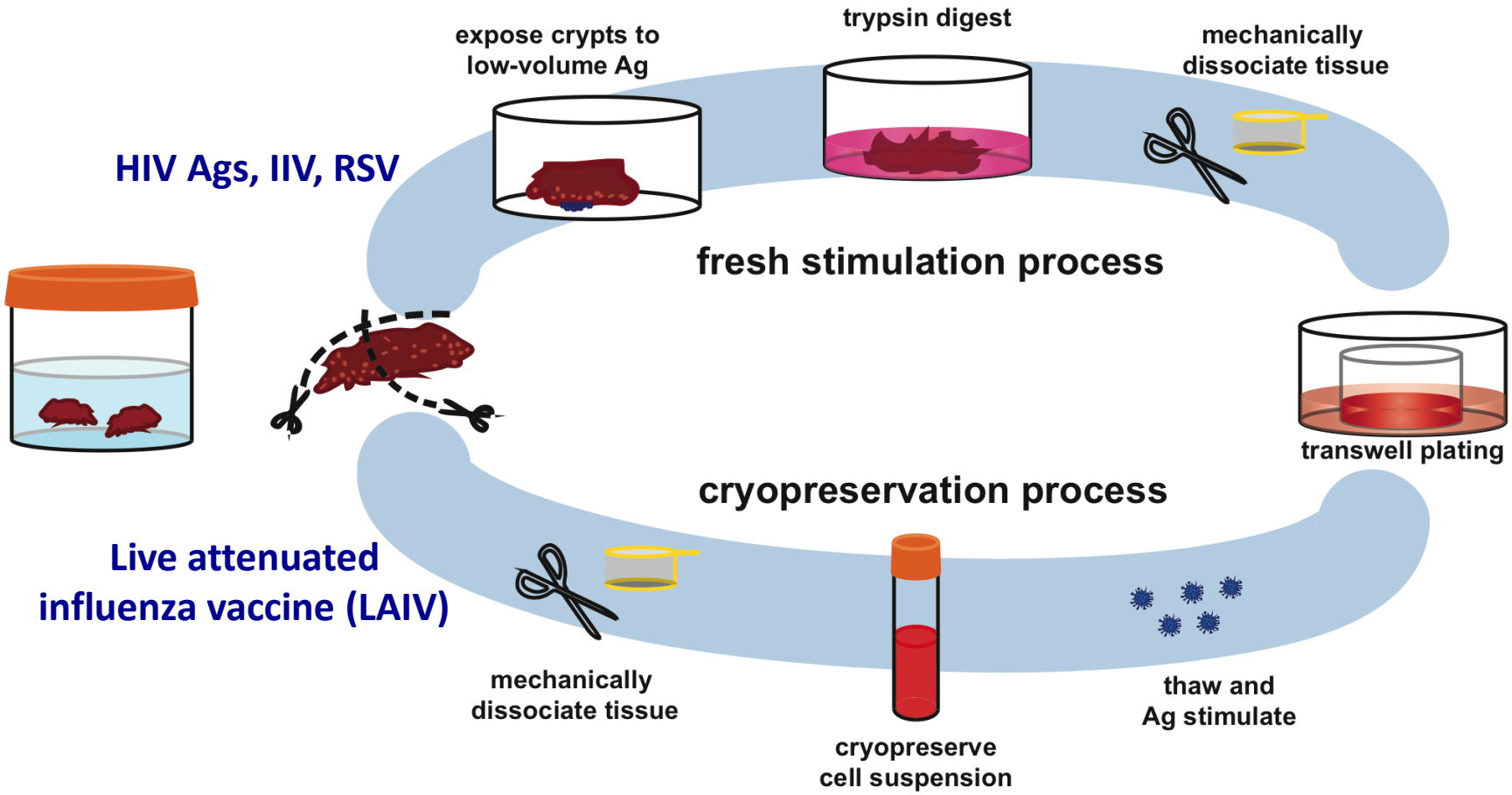
- Candidate vaccines go into clinical trials with limited opportunities to test for responses in humans
- Blood measurements can be misleading or poorly predictive of protection
- Studying the human adaptive response at the site of formation (lymphoid tissues) is challenging

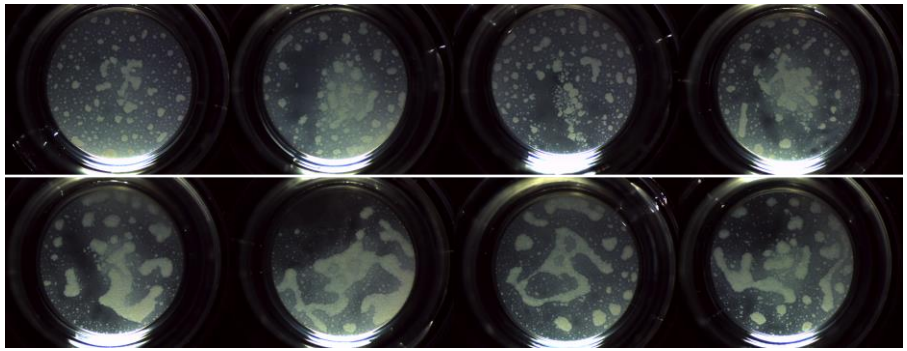
A representative model of the human adaptive immune response could bridge this gap

Organoids for human infection & immunization studies

- **Organoid**: an *in vitro* model that recapitulates the function, composition, and/or structure of *in vivo* tissue
- Murine cells have been used to make immune organoids (Purwada & Singh, Nat. Protocols 2017) but rely on matrices and cell lines
- 500 000 tonsillectomies are performed annually in the USA and much of the tissue is healthy; tonsils are a good representation of lymphoid tissue

Immune organoids enable mechanistic studies to be performed using human samples

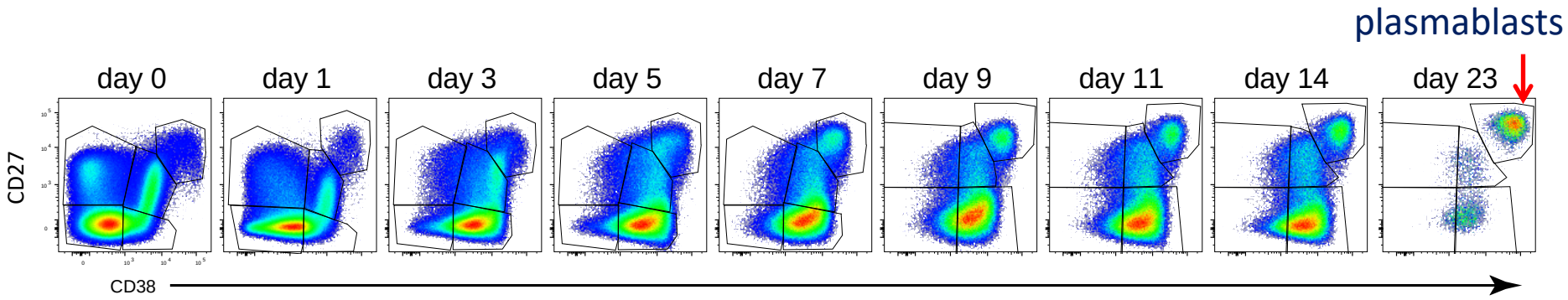


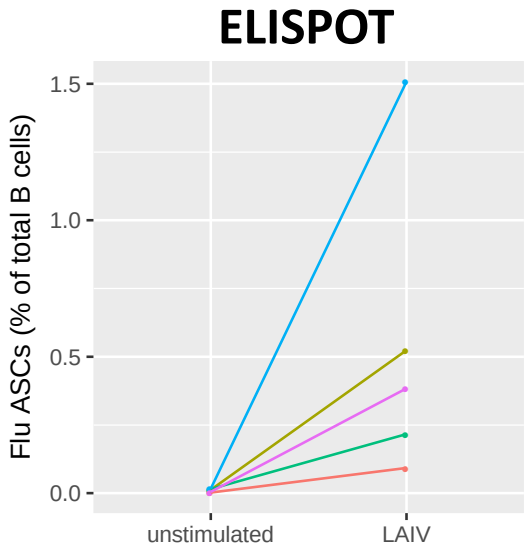


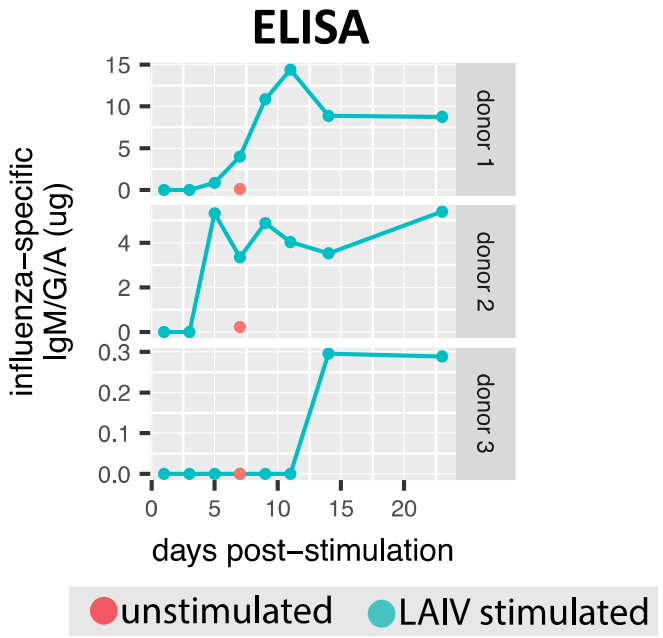
Unstimulated organoids

Live attenuated flu vaccine

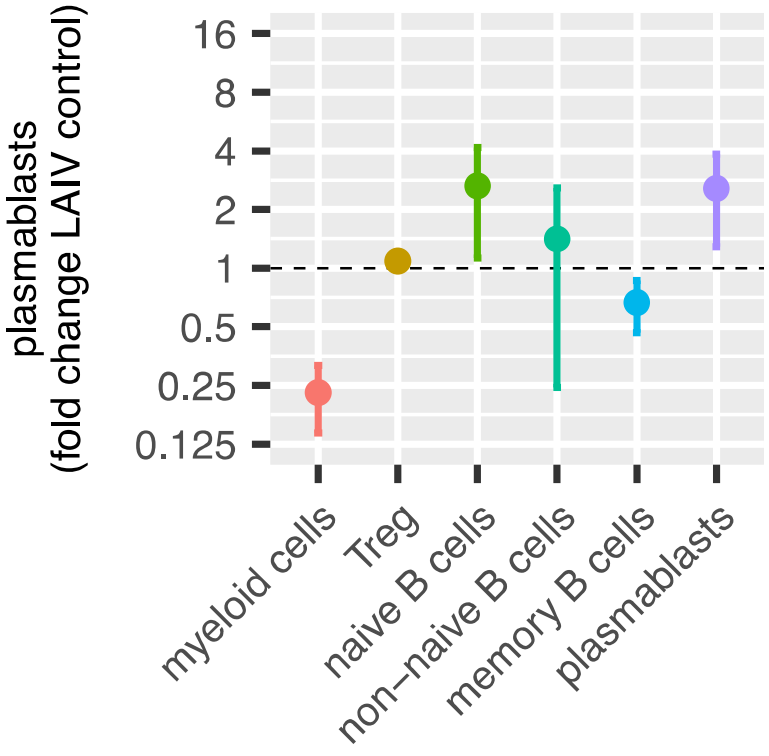
B cells differentiate in response to vaccine stimulation and make microgram quantities of specific antibody

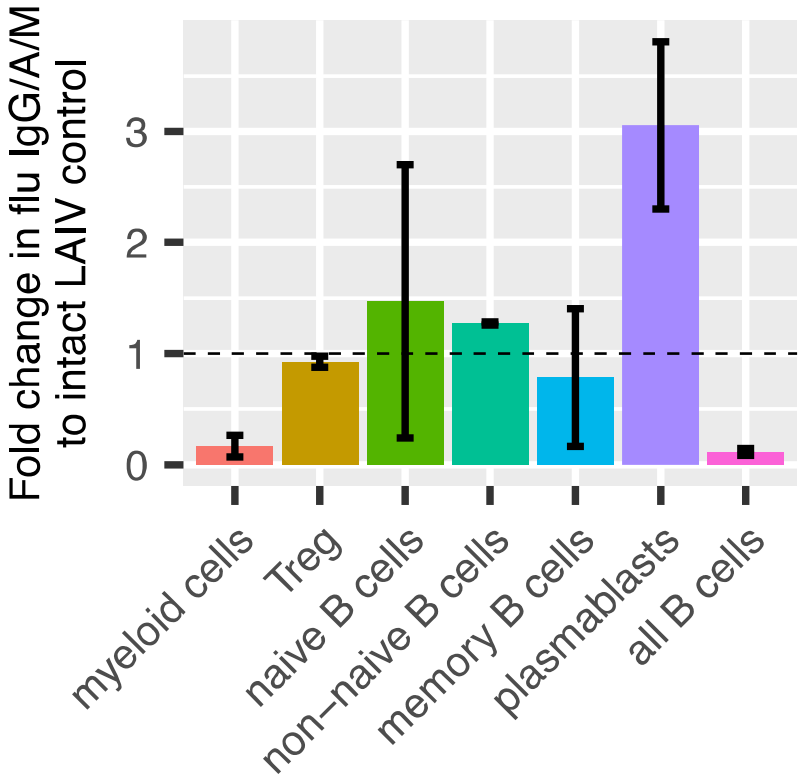




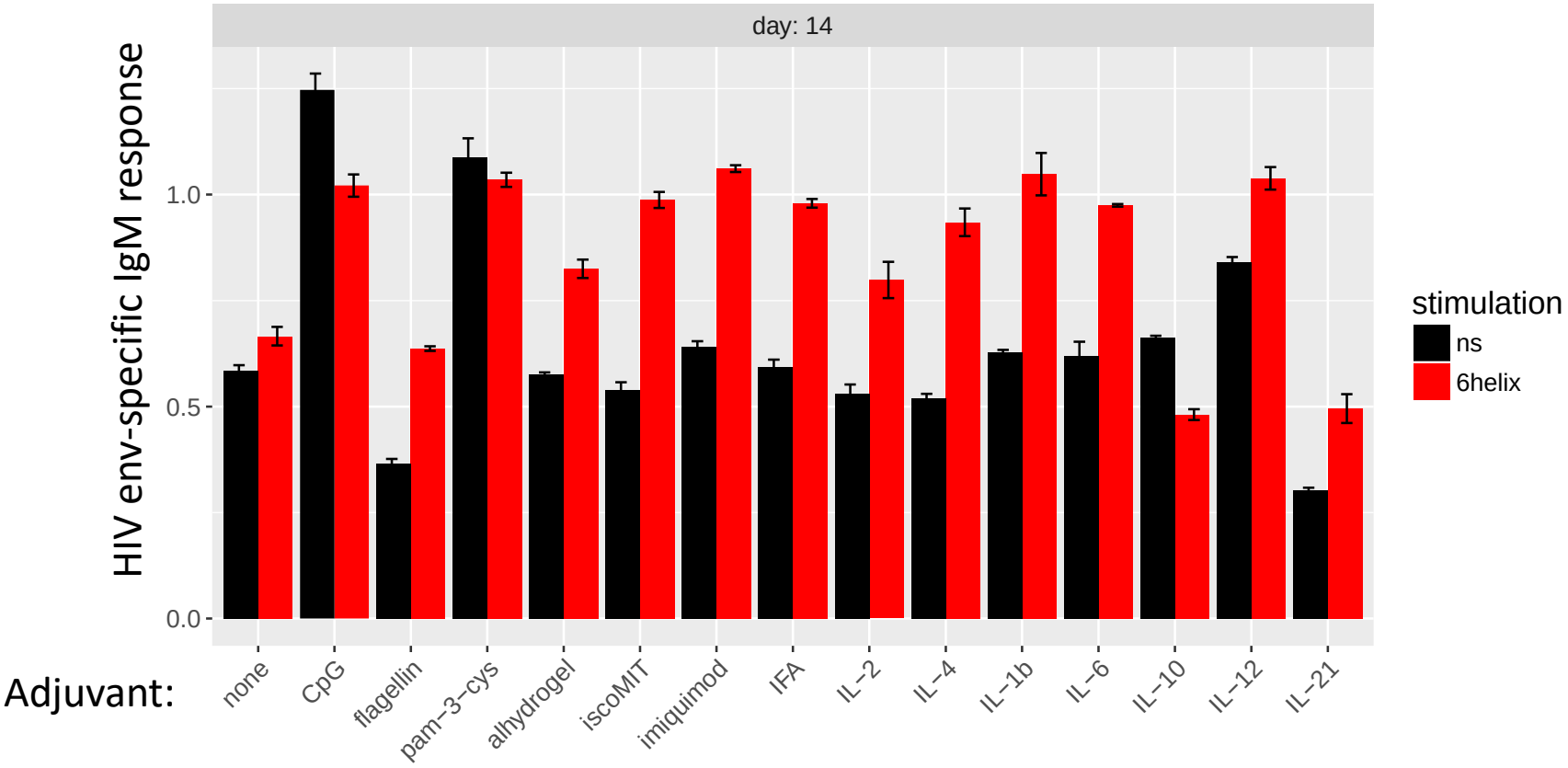


Organoids can be used to identify what cell types contribute to the response





Naive responses against HIV env variant using adjuvants



ELISA data by Michael Lyons, Peter Kim

Technology summary

- An **organized pipeline** from clinical sample collection to technology and data analysis is crucial to studying human immune responses

- **CyTOF** can be used to collect high-dimensional data that broadly covers most immune cell types
  - potential for correlates of protection

- **Single cell sequencing** reveals diversity of the T cell response and can be used to find putative T cell targets

- In vitro **organoid models** of lymphoid tissues can bridge the gap between animal models and human trials
  - probe the mechanisms of adaptive responses
  - more antigens & adjuvants under development [your vaccine here!]

Acknowledgements

Mark Davis

Huang Huang

Jacob Glanville

Arnold Han

Tom Scriba

Alex Sette

Chris Garcia

Marvin Gee

Calvin Kuo

Ameen Salahudeen

Scott Boyd

Peter Kim

Mike Lyons

Holden Maecker & team

Greg Hammer & team

Corry Dekker & team

We are grateful to the many subjects who volunteered for these studies

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