U.S. FOOD & DRUG ADMINISTRATION

Session 2: Assays to monitor innate immune activation and inflammation: technical challenges and best practices

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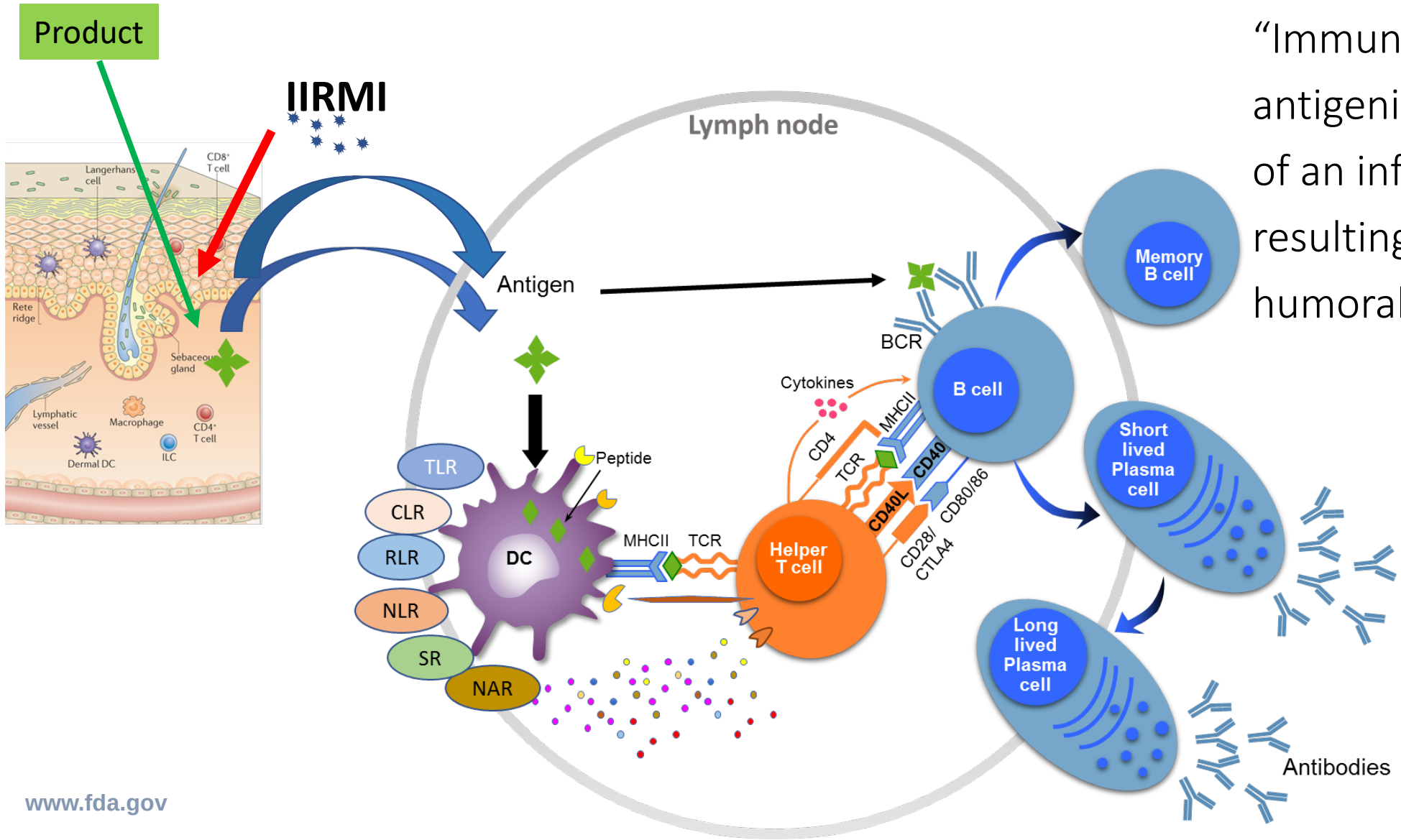
January 26, 2021

Disclaimer:

The opinions and views expressed herein are my own and not necessarily reflective of those of the FDA or current policy

APCs and Thelper Cells are the Lynchpin in Generating Immune Responses to Therapeutics Peptides

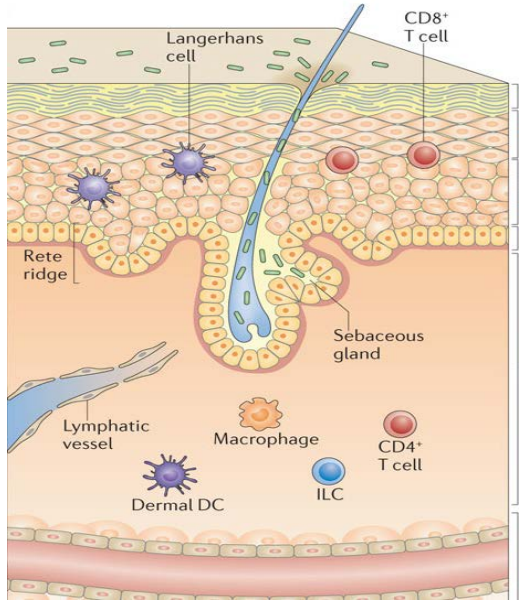


"Immunogenicity is antigenicity in the context of an inflammatory milieu resulting in a successful humoral response".

Modified from Krishna and Nadler, 2016

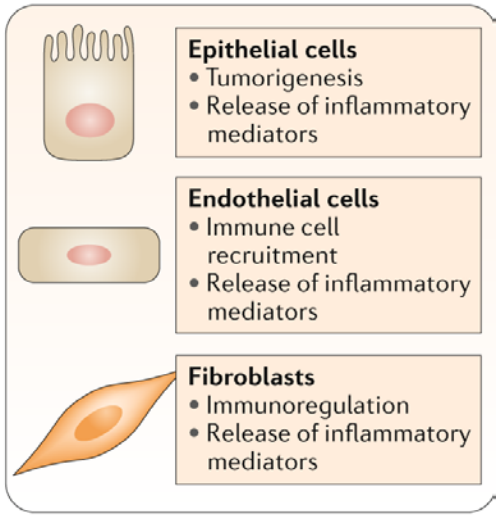
Innate immune system cells:

Immune cells

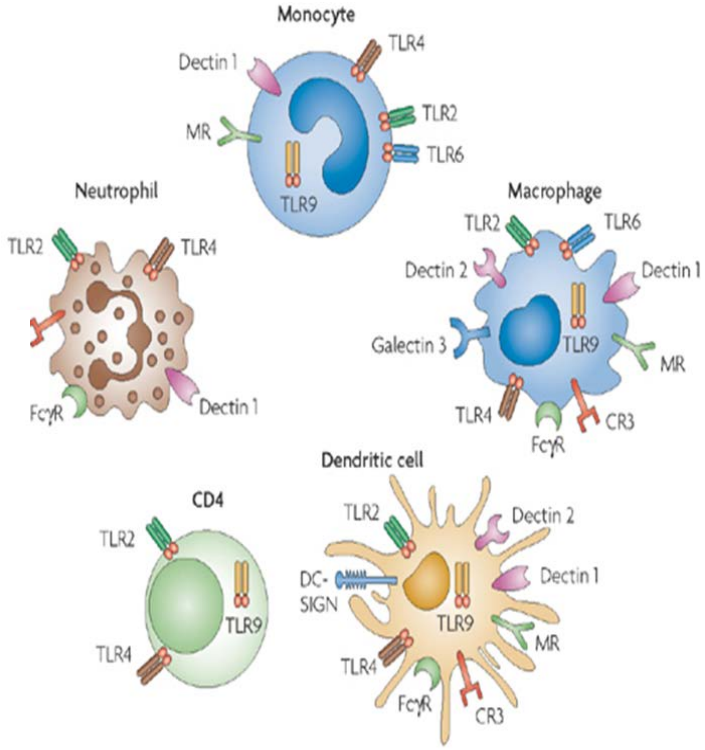


Non-Immune cells

& Basophils



PAMPs
DAMPs



Receptors:

- TLRs
- NLRs
- RLRs
- CLRs
- Nucleic Acid R
- NAMPs
- TRPs
- RAGE
- TREMs
- GPCR
- HMGB1
- Ion channels

Gong et al, Nat Rev Immunol.2020

Inflammation jump-starts the immune response

APC

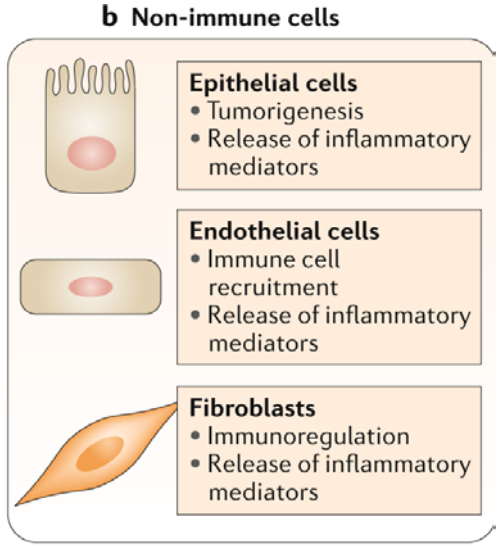
Inflammatory Mediators

Local reactogenicity/ infusion reactions

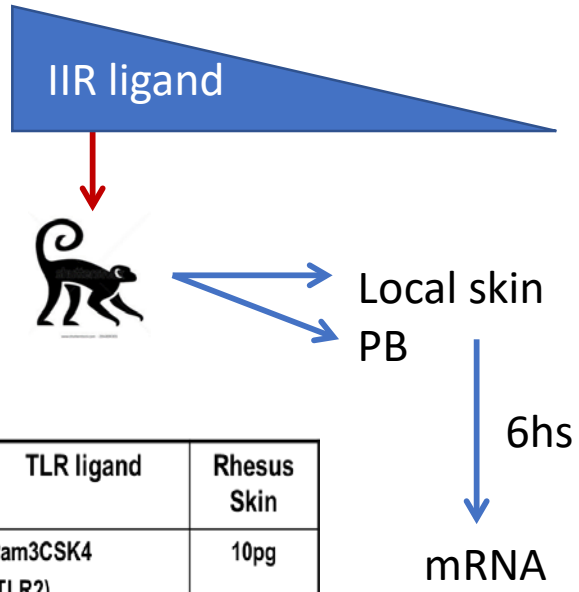
Chemotaxis → APC recruitment

APC activation

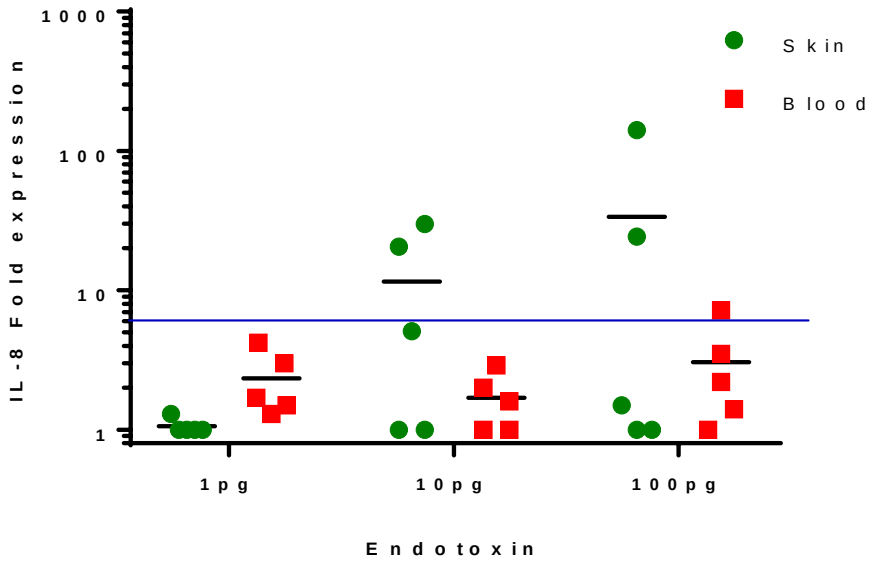
- Pino/endocytosis
- Phagolysosome fusion
- Ag processing &presentation
- Costimulatory molecules (CD83,CD86,etc)
- Cytokines->Type of T cell response



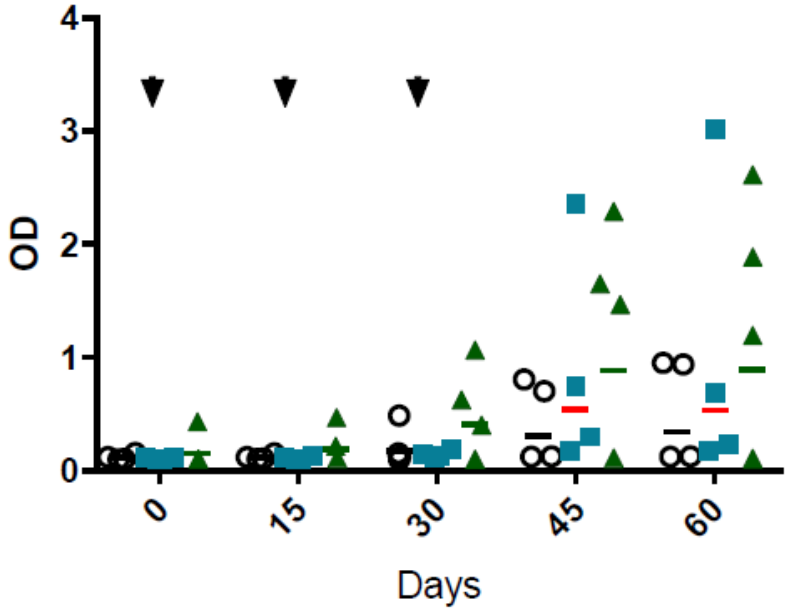
Trace levels of Innate Immune Receptor (IIR) ligands induce local immune activation and increase product immunogenicity in macaques



TLR ligand	Rhesus Skin
Pam3CSK4 (TLR2)	10pg
Poly I:C (TLR3)	1ng
Endotoxin (TLR4)	10pg
Flagellin (TLR5)	1ug
FSL-1 (TLR2-6)	10ng
CLO75 (TLR7)	10ng
CpG ODN (TLR9)	5ug
B glucan (dectin1)	10ng
MDP (NOD2)	1ng



Abs to Rasburicase



Low levels of IIRMI increase local expression of pro-inflammatory genes and act as adjuvants

Haile et al, 2017

Innate Immune Response Modulating Impurity (IIRMI) assessment strategy

Inn. Imm. Receptors
- TLRs
- NLRs
- RLRs
- CLRs
- Nucleic Acid R
- NAMPs
- TRPs
- RAGE
- TREMs
- GPCR
- HMGB1
- Ion channels

- IIRMs are trace level of product or process related impurities or contaminants that can activate the innate immune system facilitating an immune response.
- Complete spectrum of potential IIRMI is unknown
- Converging signaling pathways that lead to cell activation and cytokine secretion

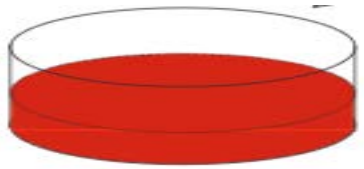
Monitor their immunomodulatory activity using sensitive cell-based assays

In Vitro Assays for Innate Immune Responses*

PBMC
Whole blood
Reporter Cell lines

DS
DP
Peptide impurities

Suitability controls (TLR agonists)



Incubate

- NFκB activation
- Cytokine production
- Cell marker expression
- mRNA expression patterns

* Other methods capable of identifying IIRMI may be used; discussed with the Agency.

Critical Assay attributes:

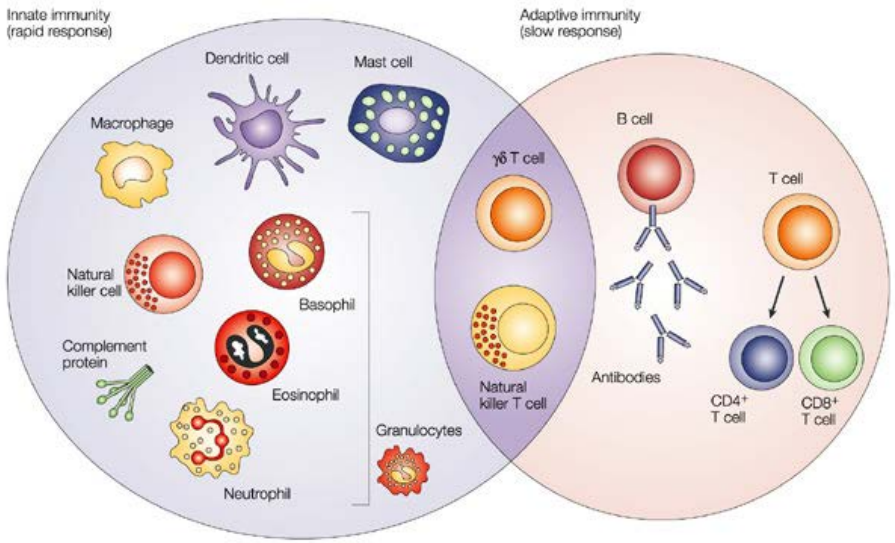
- Selection of assay platforms.
  - 1ry vs cell lines
- Establishing Drug Tolerance, Assay Sensitivity, and Reproducibility
  - Cell viability (cell-dependent)
  - Optimal product dilution
  - Matrix interference
    - Drug formulation
- Suitability controls
  - Selection of Neg., Low, High controls
- Readouts:
  - Single vs multiple readouts
  - Assay timing
- Result interpretation
  - Comparative qualitative and quantitative assessment of innate immune activation in comparison with RLD, not a positive/negative determination based on a cut point.

Assay attributes: Cell Platform

Cell line	Origin	Commercial	Considerations
Whole blood (fresh)	Human, PMN, Ly's, Monos. Minimal Mø, DC, pDC, Mas, Eos, Bas etc.	Yes-difficult	- Donor qualification - Variability - Sample processing
PBMC (fresh or frozen)	Human Ly's & Monos. Minimal PMN, Mø, DC, pDC, Mas, Eos, Bas etc.	Yes	
RAW-BLUE	Mouse macrophages	Yes	- Limited PRR array - Low sensitivity to DP aggregates - Not PRR specific
Macrophage-like-MonoMac6 (MM6)	Human monocytic cell	Yes	
THP-1	Human monocyte	Yes	
HEK 293-Receptor	Human embryonic kidney	yes	- PPR specific

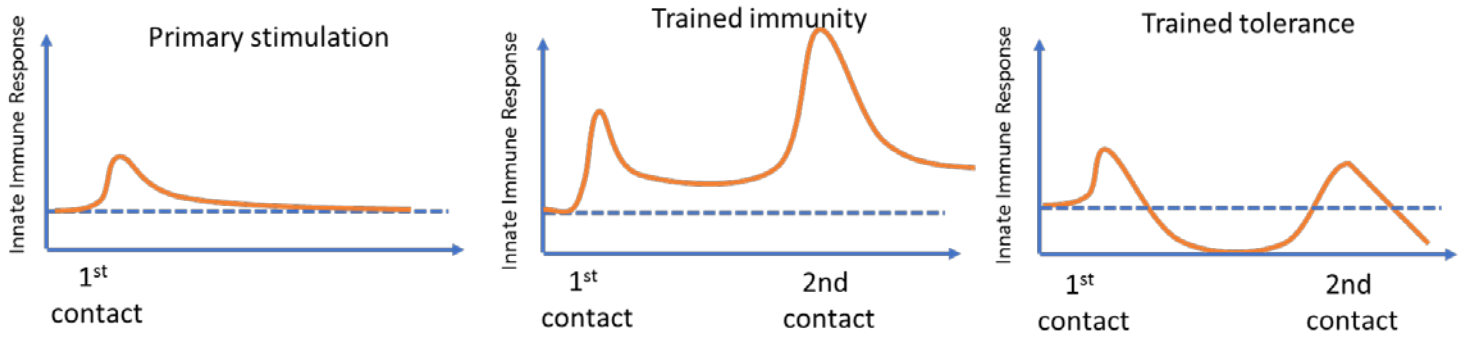
Assay attributes: Number of donors needed when using PBMC or whole blood:

- Innate immune receptors are highly conserved
- Donor to donor variations
- Trained innate immunity



Adapted from Dranoff 2004

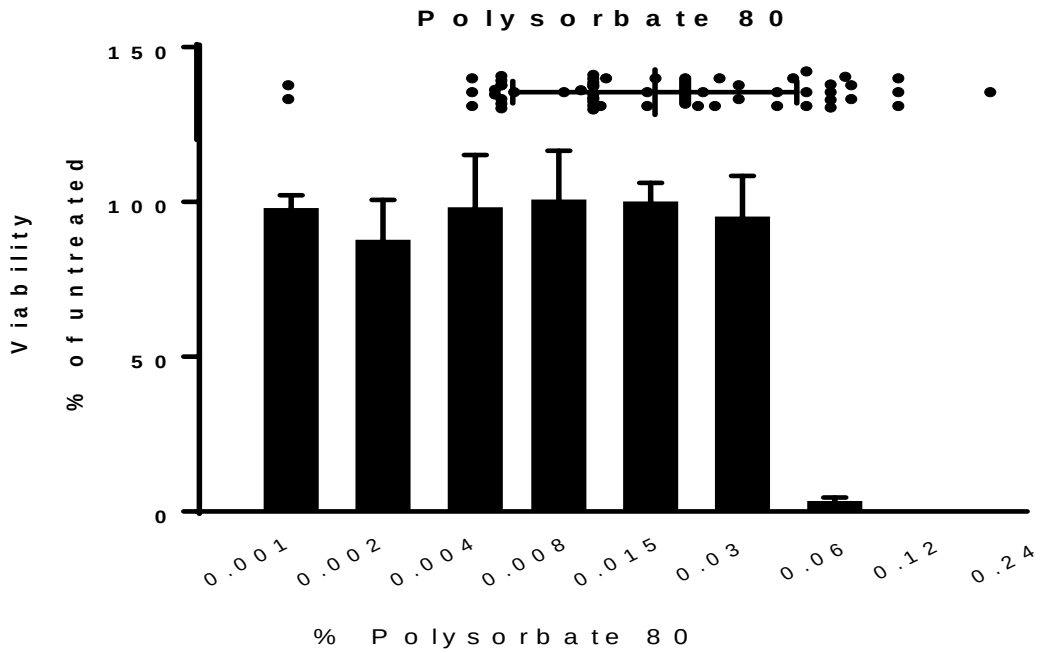
Adaptive Immunity: memory
Innate Immunity: training

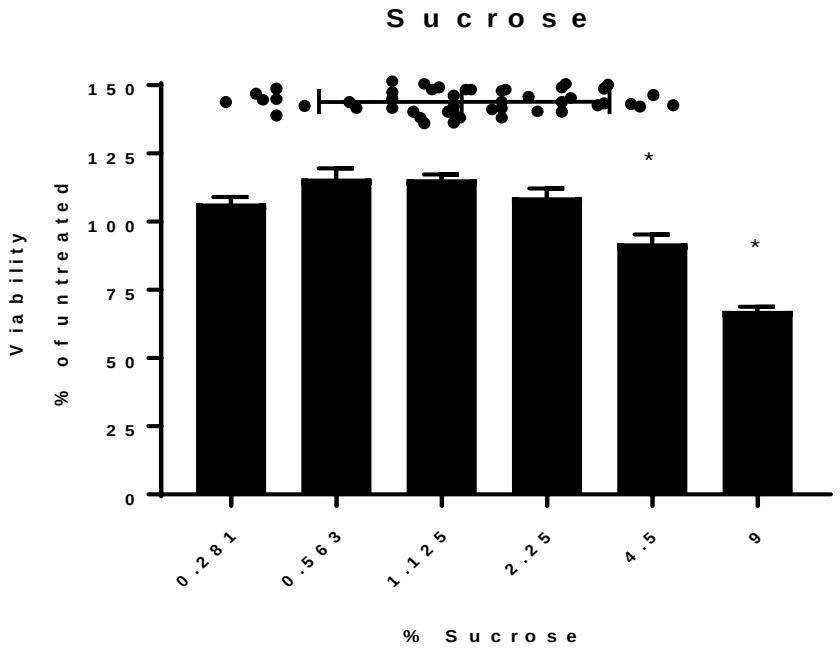


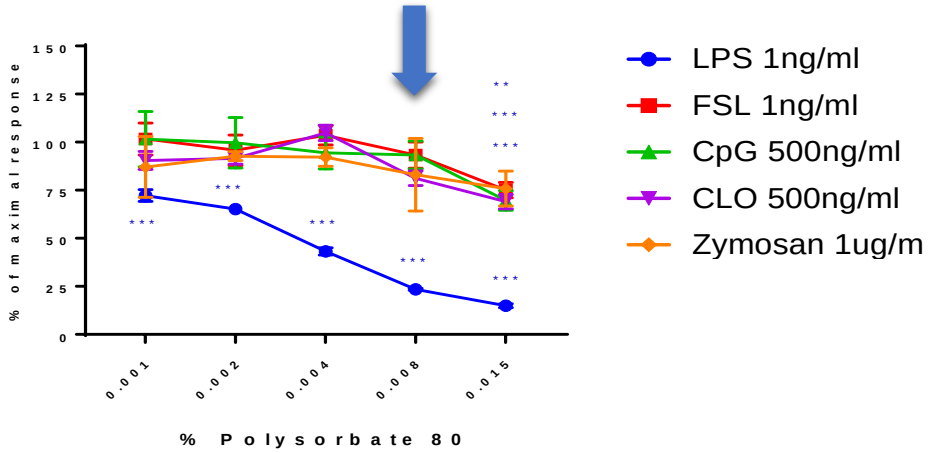
Mechanisms of trained immunity:

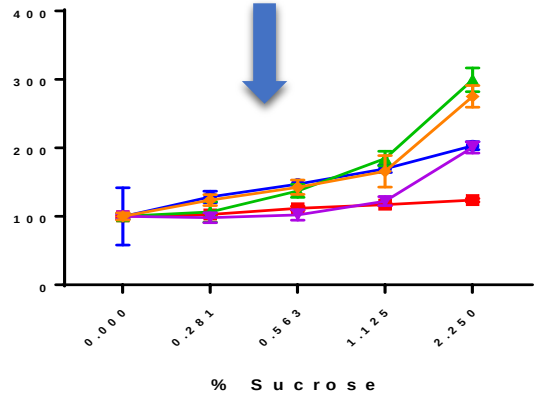
- Skewing of myelopoiesis
- Transcriptional / epigenetic changes
- Metabolic reprogramming
- Transcriptional signaling

Assay attributes: Product formulation and IIRMI masking





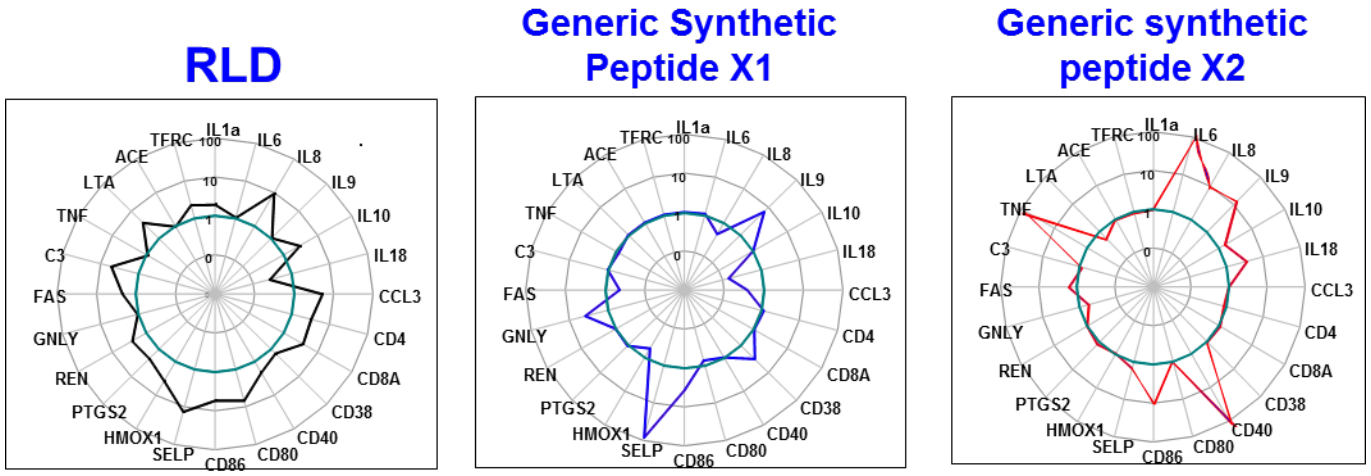


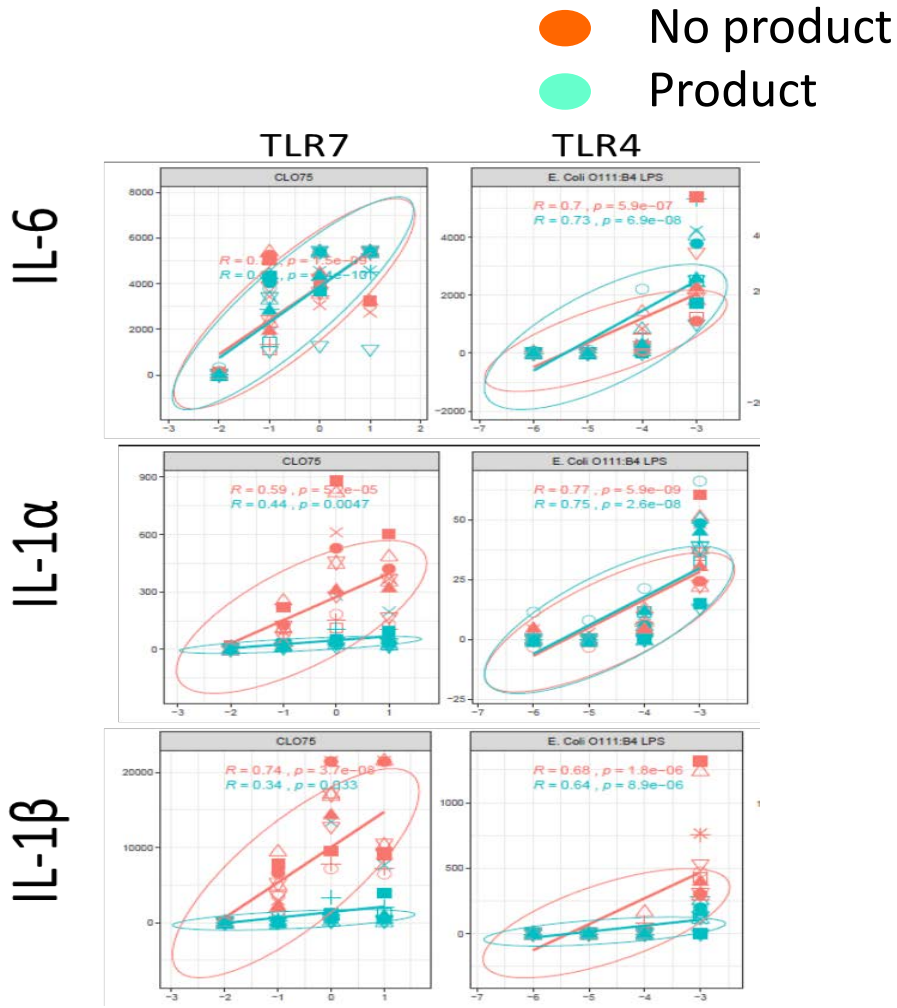


Seth Thacker, in prep.

Assay attributes: Readout and interpretation

- Single vs multiple readouts
  - Transcription markers (NFκB)
  - Cytokines
  - Cell surface markers
  - mRNA arrays





Assess immunogenicity risk: last thoughts

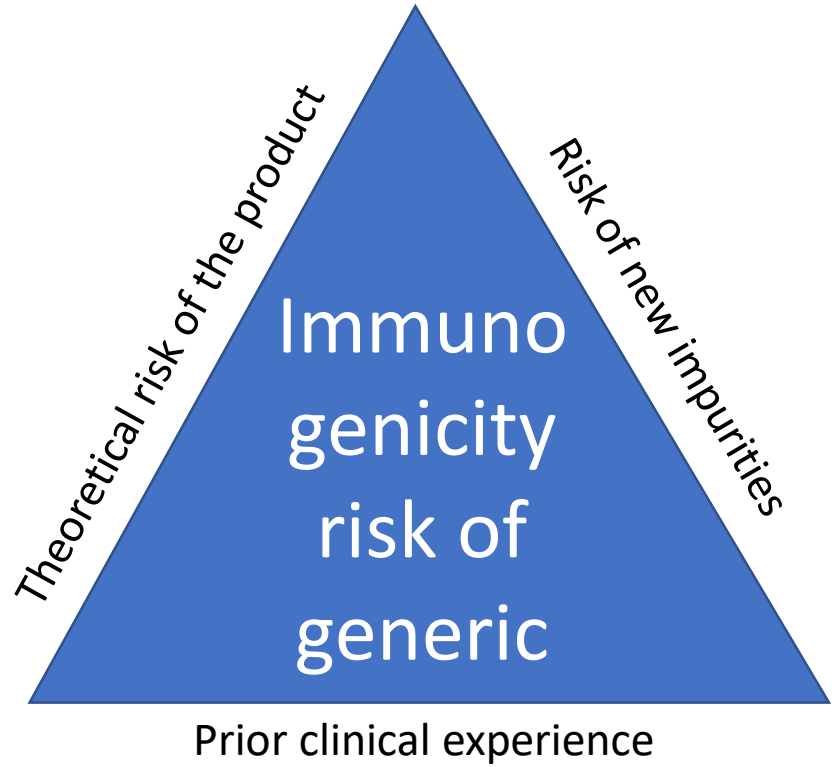
- Need for careful validation of these assays to provide meaningful data.
- Evolving technology and the need for the scientific community to compare the performance of tools or techniques.

Acknowledgements

Contributing lab members on this project (current and past):

- Seth Thacker
- Cheng Her
- Logan Baker
- Lydia Haile
- Eduardo Mufarrege
- Montserrat Puig
- Swamy Polumuri
- Vivian Wang
- Mohanraj Manangeeswaran
- Derek Ireland

- Susan Kirshner, Review Chief
- Amy Rosenberg, Division Director
- Eric Pang



The experts: Jeremy Fry

- Director, ProImmune Ltd., Oxford, UK. He developed the MHC Pentamer system to monitor antigen-specific CD8+ T cells, and currently directs a group focused on developing and implementing assays to measure wanted and unwanted immune responses and assess immunogenicity risk. He will talk about his experience developing and performing whole Blood Cytokine Release Assays to Assess the Risk of Innate Immune Activation to Generic Peptide Products.

The experts: Marina Dobrovolskaia

- Director of Operations and the Head of Immunology Section at the Nanotechnology Characterization Laboratory (NCL). Dr. Dobrovolskaia leads a team responsible for the design, development and validation of bioanalytical ligand-binding assays to support pharmacokinetic and toxicity studies in a variety of drug development projects. She will present her studies to assess IIRMI in teriparatide using at PBMC platform.