Development of a human in vitro T-cell dependent antibody response assay

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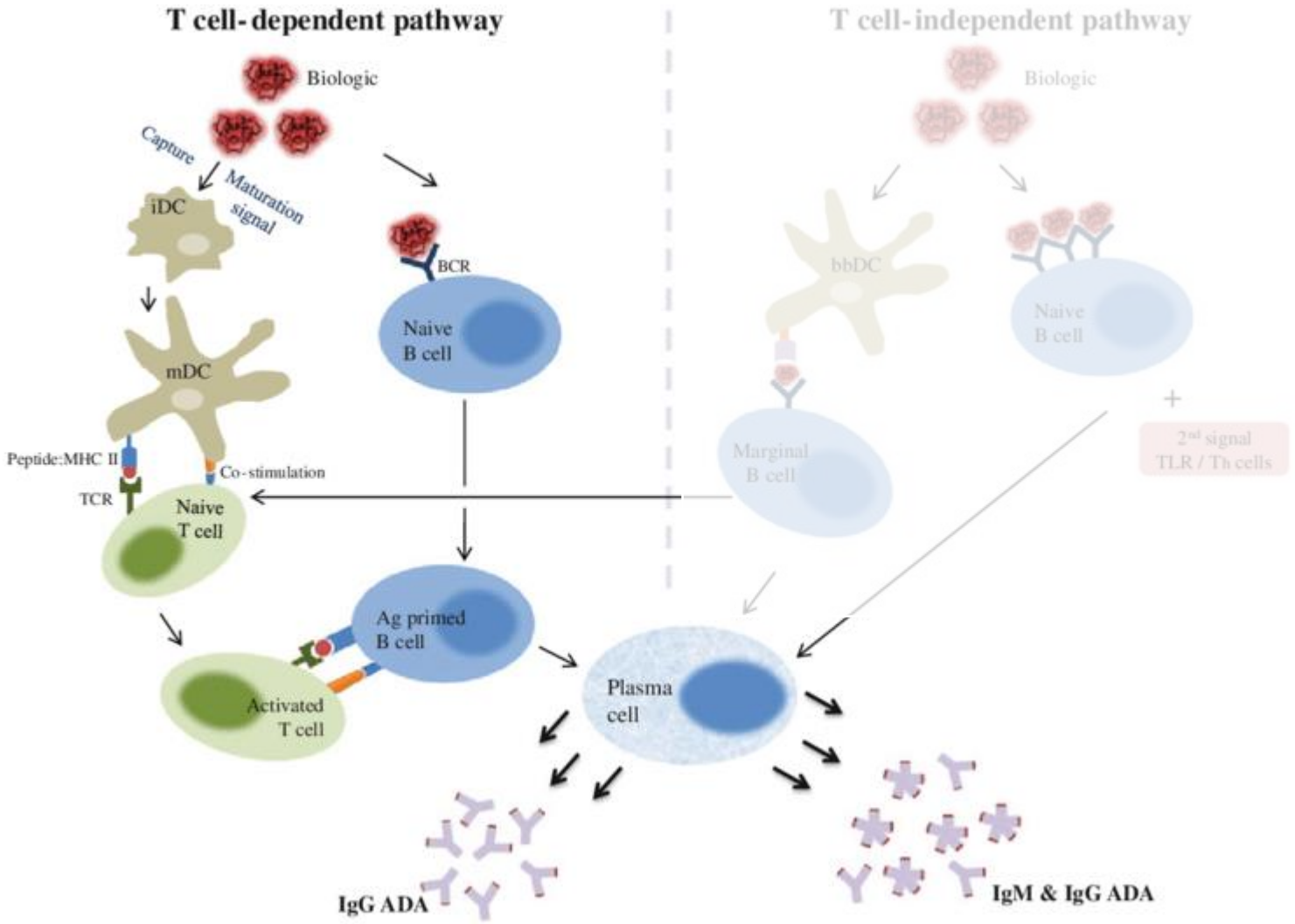
ECETOC Workshop on Immunotoxicity Assessment - 9 July 2025

Grow. Learn. Discover.

T cell-dependent antibody response (TDAR) response *in vivo*



Lymph node models

Preclinical tests

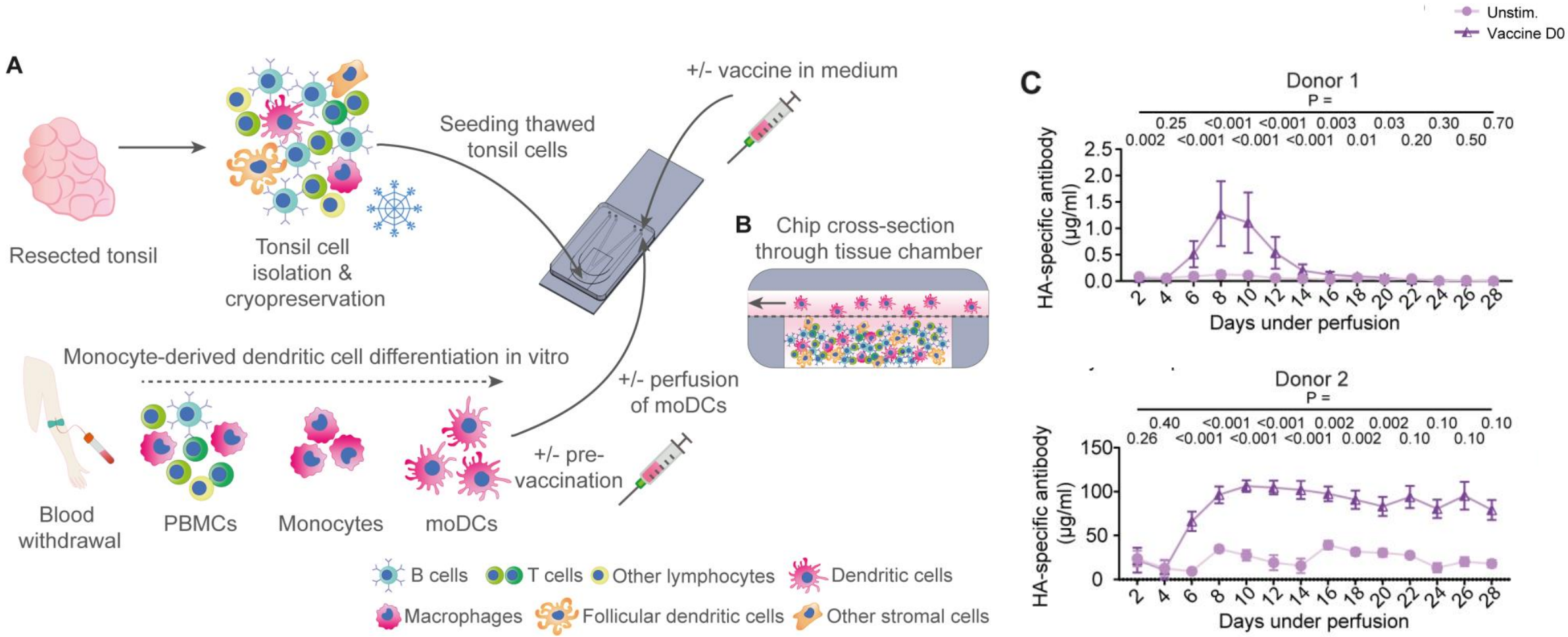
	2D models	3D models	Animal models	Human clinical trials
Production method	Cells grown on a rigid and flat surface	3D scaffold that resembles ECM	In vivo natural structure	In vivo natural structure
Cell type	Depends on model type	Multitype	Highly diverse cell types	Highly diverse cell types
Natural cell morphology	No	Yes	Yes	Yes
Tissue architecture	Absent	Complexity based on design	Naturally present	Naturally present
Vascularization/perfusion	Absent	Present according to model type	Present	Present
High-throughout screening	Medium to high	Low to medium	No	No
Biobanking	Yes	Yes	Yes, only at the cellular level	Yes, only at the cellular level
Fidelity to human processes	Oversimplified, non physiological conditions	More physiological conditions	Species-specific differences	High fidelity
Costs	Low	Moderate	Moderate	High
Bioethical concerns	No	No*	Moderate	High

Morrochi *et al.*

In vitro TDAR model why?

1. Human-Relevant Immune Function Assessment
2. Predictive for Immunosuppression
3. In Vitro Alternative to Animal TDAR Assays
4. Mechanistic Insights
5. Regulatory and Safety Assessment
6. Scalable and High-Throughput Compatible

Lymphoid-tissue-on-chip



[Lymphoid-tissue-on-chip recapitulates human antibody responses in vitro | bioRxiv]

NC3Rs CRACK-IT TDAR Consortium

MIMETAS

ImmunXperts
a Q² Solutions Company

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NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

Kate Harris

BAYER

Pascale Buchmann

NOVARTIS

Hannah Morgan

MERCK

Phil Hewitt

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Nikki Marshall

Optimization, Validation, Automation and Standardization of TDAR on-a-chip for the assessment of human T cell dependent antibody responses

Translating *in vivo* to *in vitro* model

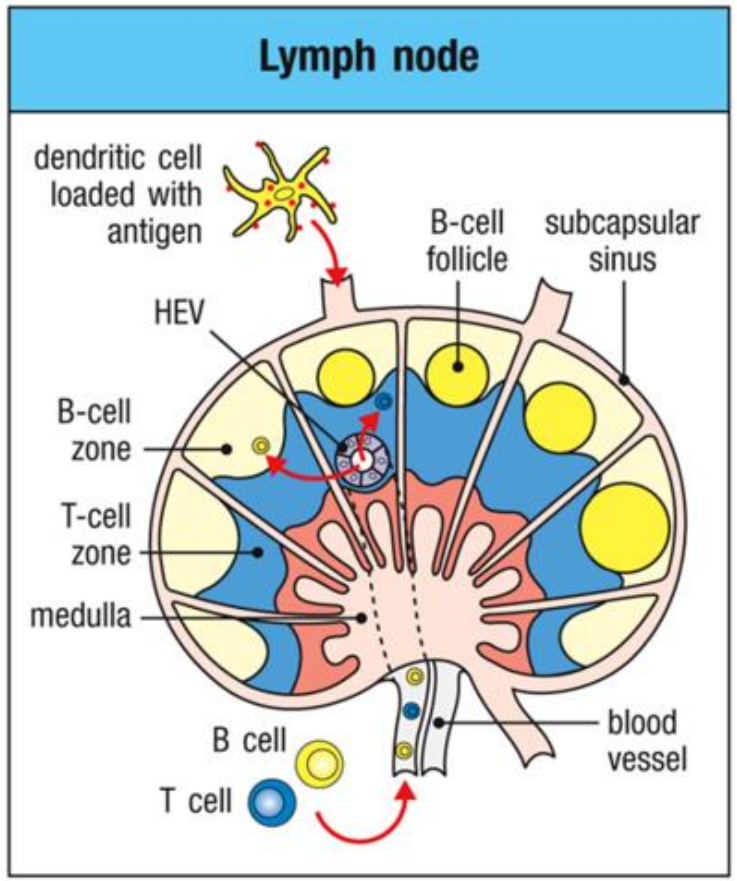
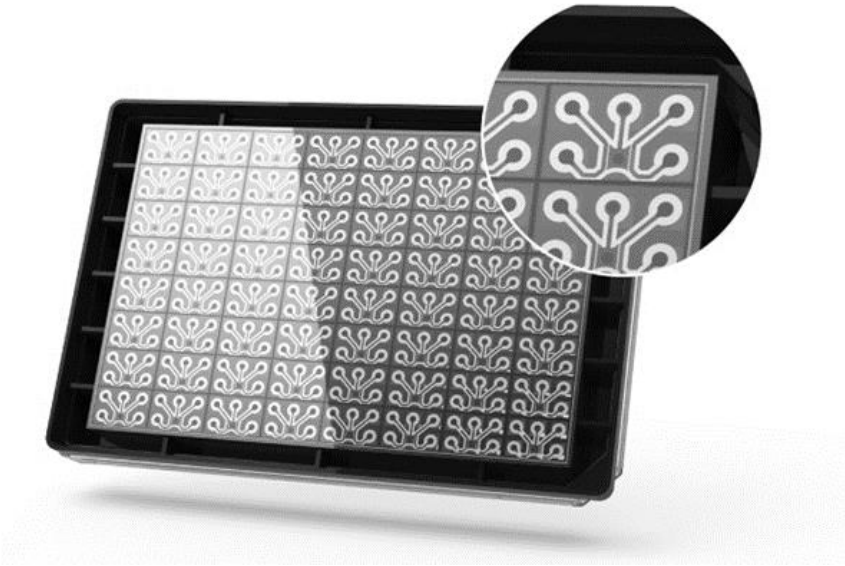
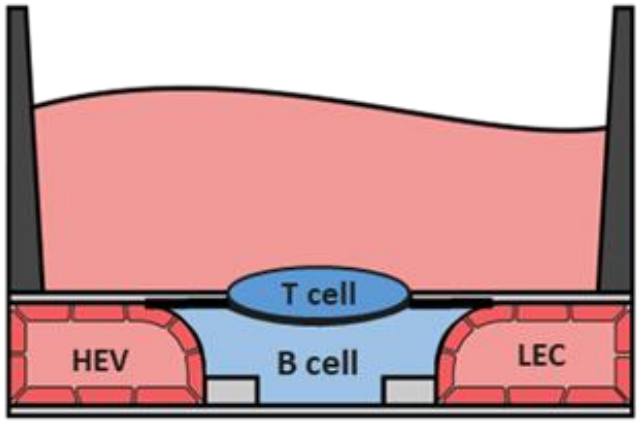


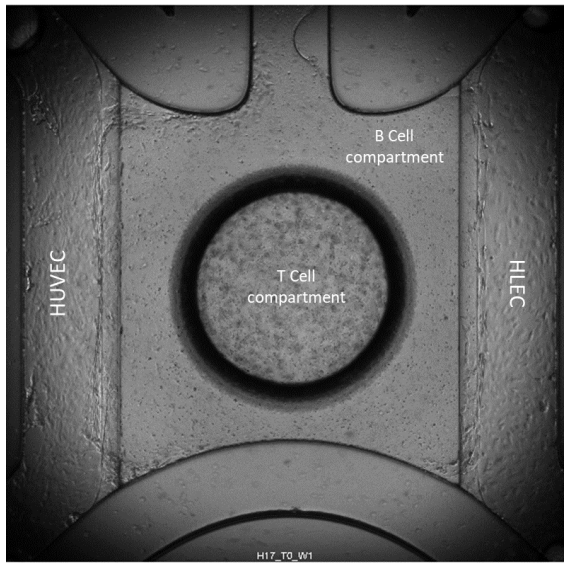
Figure 9.1 (part 1 of 3) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)



Microfluidic platform OrganoPlate® Graft
- 64 chips per plate

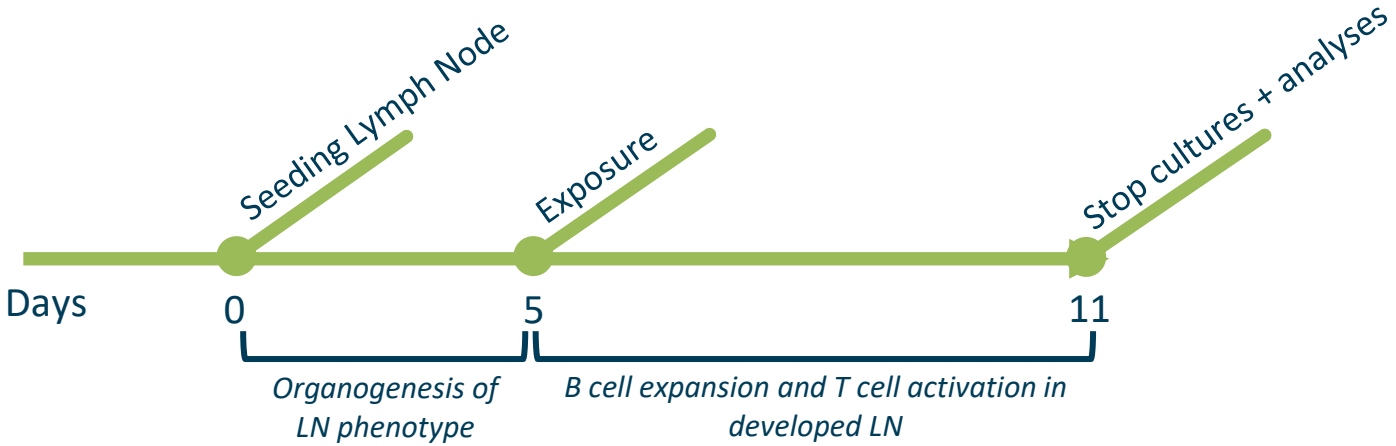
TDAR Lymph node-on-a-chip model





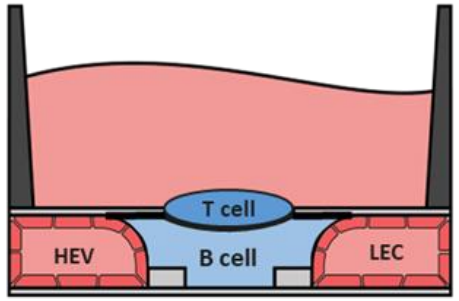
Read outs:

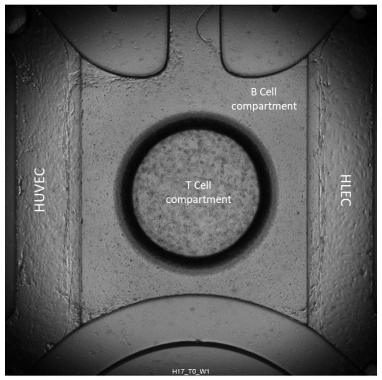
- Off plate
  - Flow cytometry
  - Fluorospot
- Supernatant
  - ELISA /Luminex
- On plate
  - Immunofluorescent staining



Application recall response

Cell configuration





N_2 frozen PBMCs (COVID exposed, ImmunXpert)

Iteration

- 4 independent experiments
  - Iteration 1 & 3 are done with donor 001
  - Iteration 2 is done with 002
  - Iteration 4 donor 003 pre-covid (only IF stainings) & donor 003 post-covid

Exposure conditions

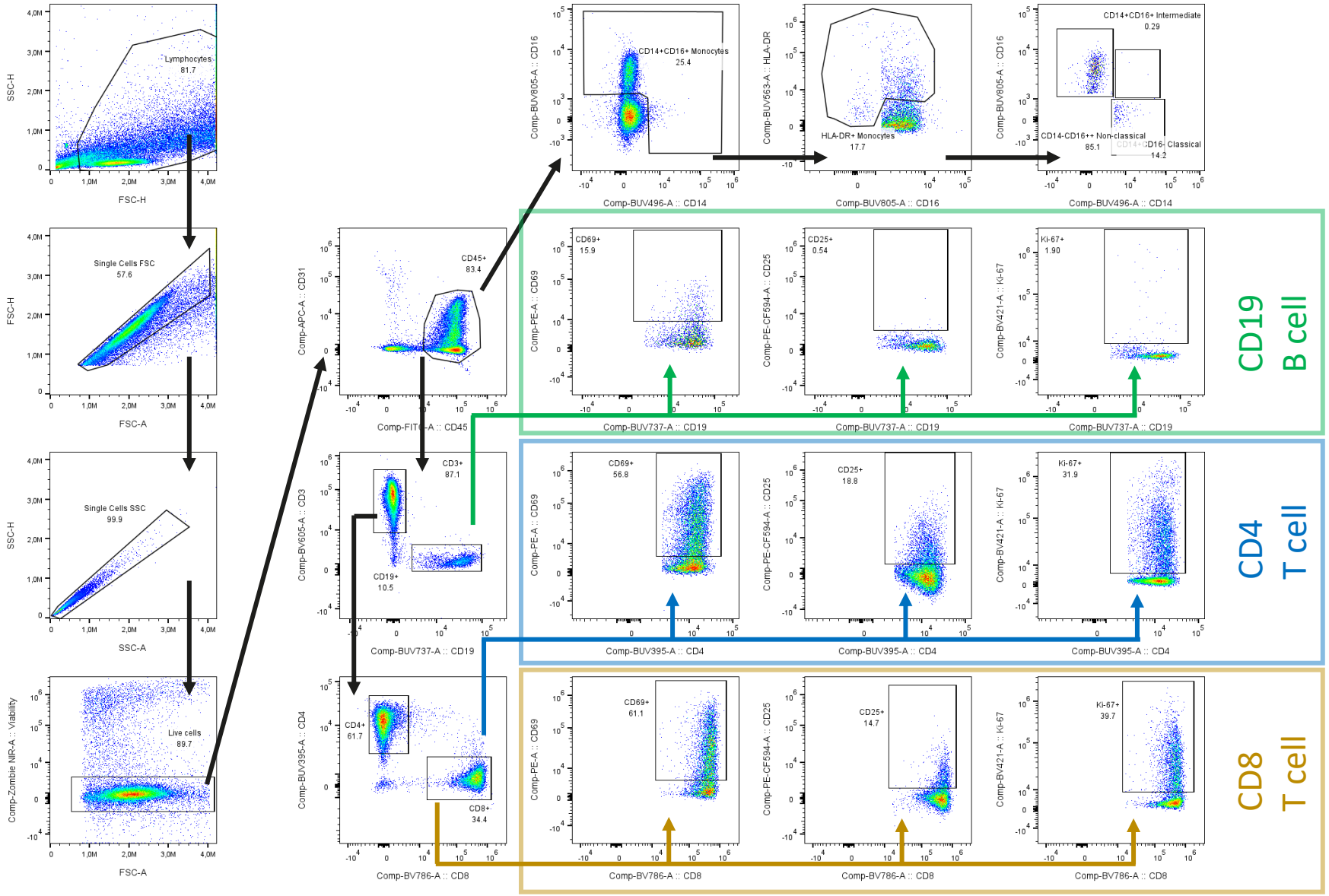
- No stimulus
- SARS-CoV-2 spike protein
- R848+IL-2 exposure

- 1st exposure day 5
- 2nd exposure day 8

Readouts

- Spectral flow cytometer 5-laser
  - 14-color antibody panel (Extracellular activation markers: CD69, CD25: Maturation markers: CD86, CD138; Intracellular proliferation marker Ki-67)
- Immunofluorescent staining
- Fluorospot
  - Total IgG
  - SARS-CoV-2 specific IgG

Flow cytomery: Unstimulated condition – gating strategy

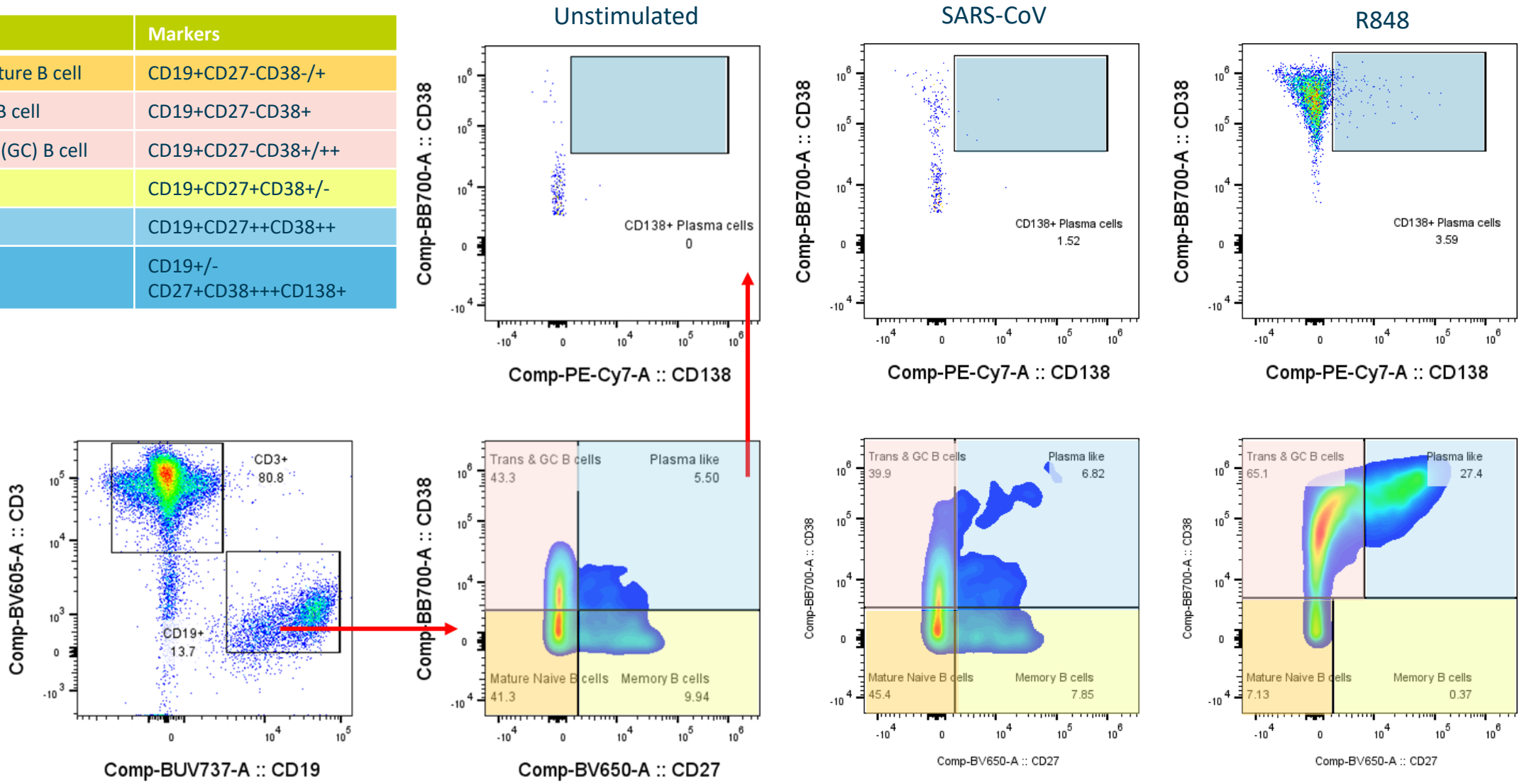


At day 11 of culture cells could be isolated from 3D lymph node and analyzed by flow cytometry

- Viability was above 89% for all three conditions
- Possible to separate different immune populations (eg B cells / T cells) and look at status

B cell frequencies in in vitro 3D lymph node

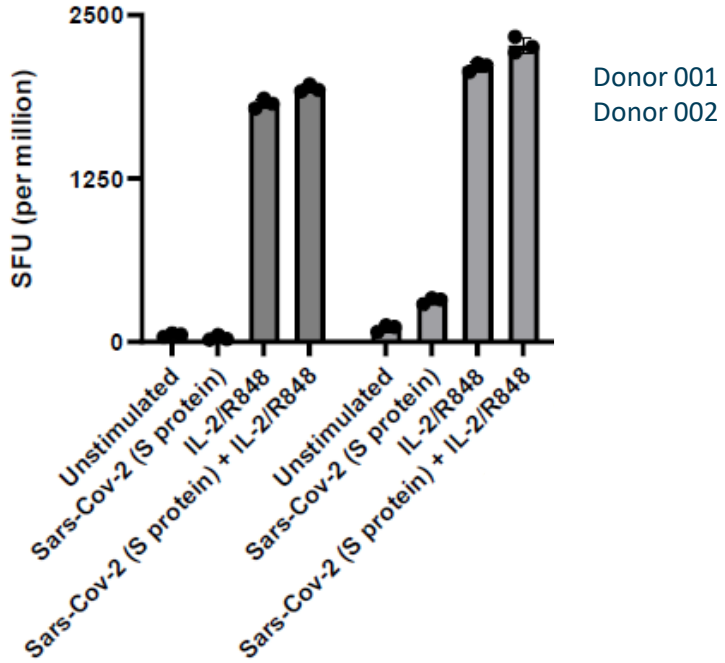
Phenotype	Markers
Naïve B cells/mature B cell	CD19+CD27-CD38-/+
Transitional (Tr) B cell	CD19+CD27-CD38+
Germinal Center (GC) B cell	CD19+CD27-CD38+/++
Memory B cells	CD19+CD27+CD38+/-
Plasmablast	CD19+CD27++CD38++
Plasmacell	CD19+/- CD27+CD38+++CD138+



- Upon addition of SARS-CoV also a switch in B cell types is observed, but is less clear compared to R848

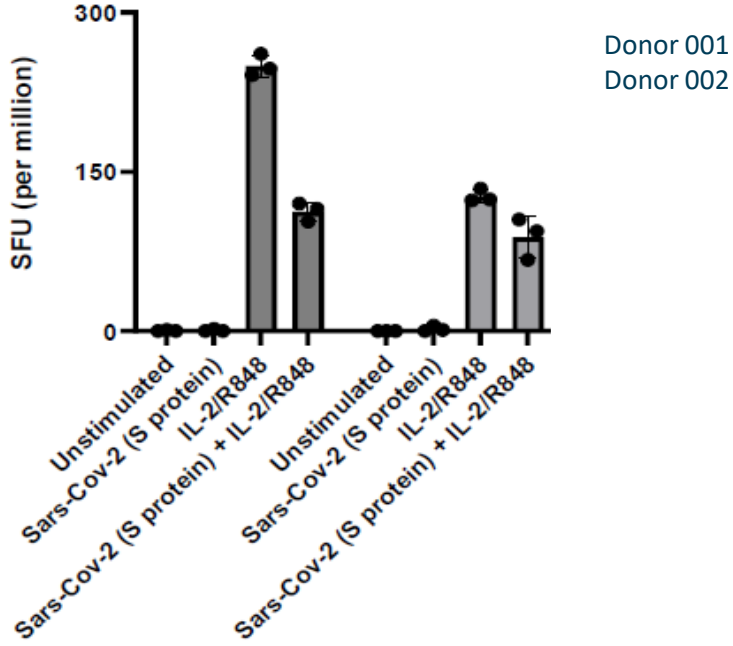
Fluorospot results 2D: ImmunXperts

Total IgG



2D assay with frozen PBMCs showed increased total IgG production upon IL-2/R484 stimulation. Small increase in total IgG in 1 donor upon Sars-Cov-2 protein

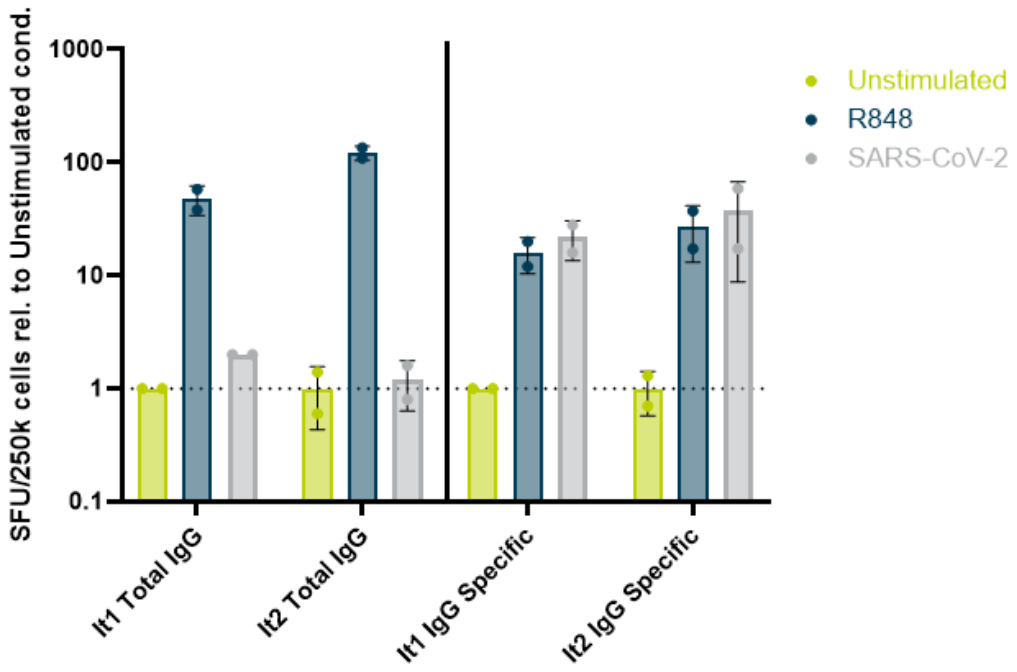
Specific IgG



2D assay with frozen PBMCs showed specific IgG production upon IL-2/R484 stimulation. No specific IgG production in upon Sars-Cov-2 protein stimulation

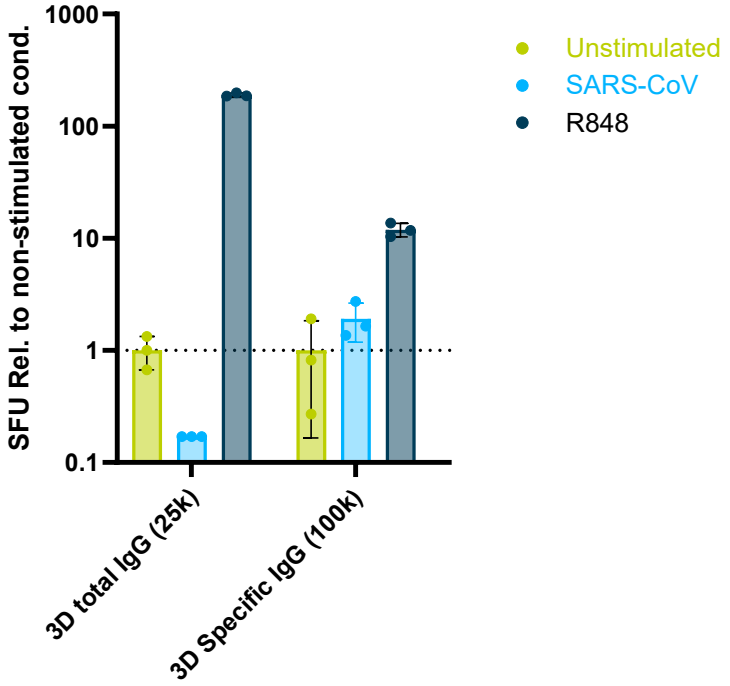
Detection of antibody production in in vitro 3D lymph node

Specific antibody production after SARS-CoV-2 exposure in it 1 and 2

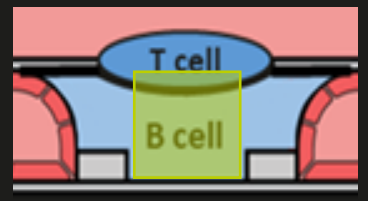


This indicates that memory B cells in the 3D lymph node respond specific to SARS-CoV-2 stimulation without activating complete lymph node

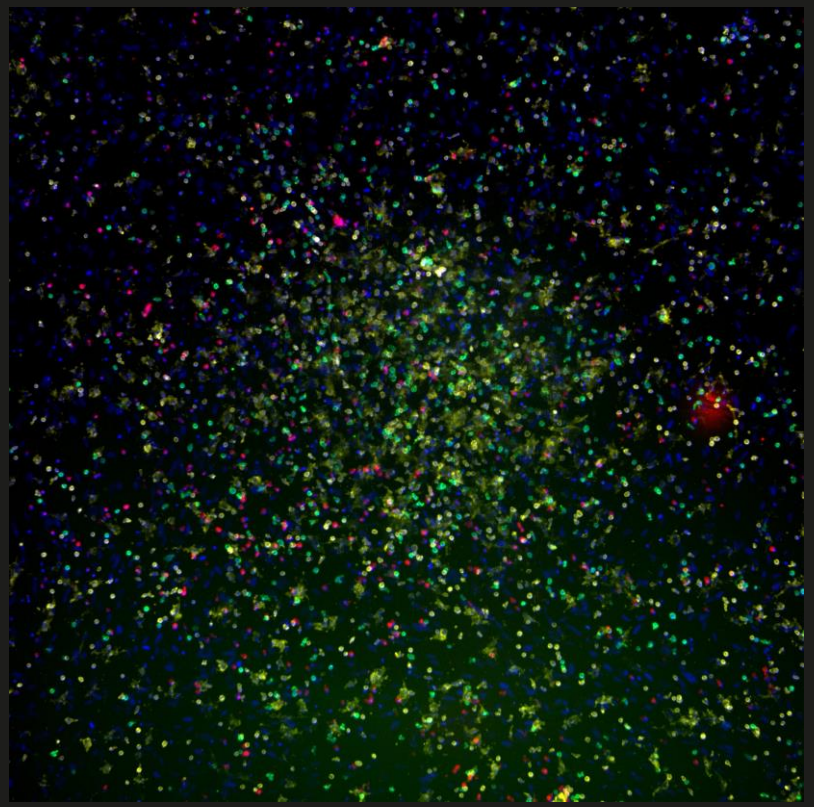
No specific antibody production after SARS-CoV-2 exposure in it 3 and 4



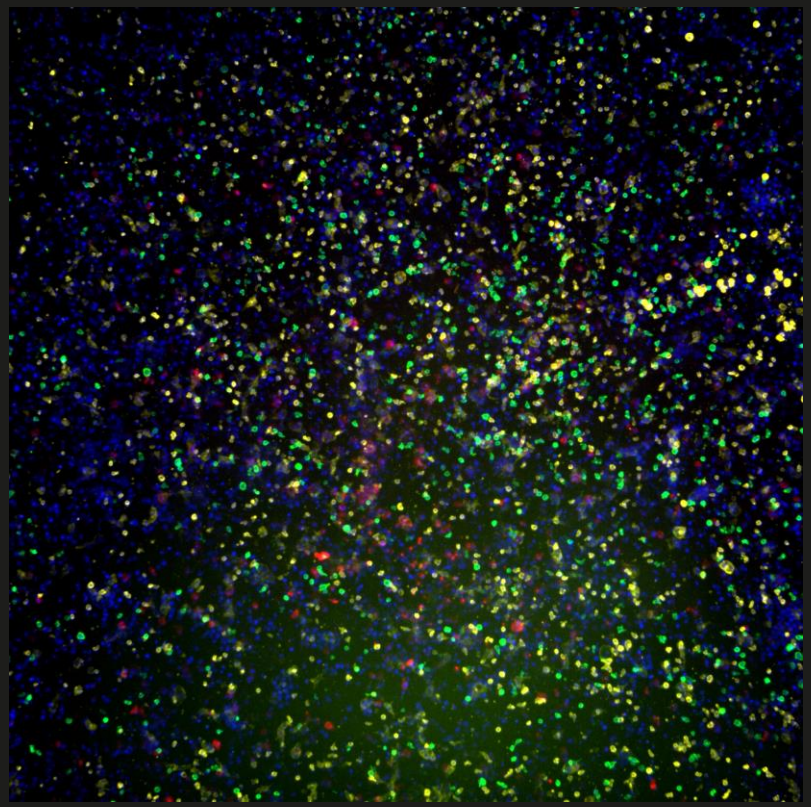
Immune cells in 3D lymph node – post covid



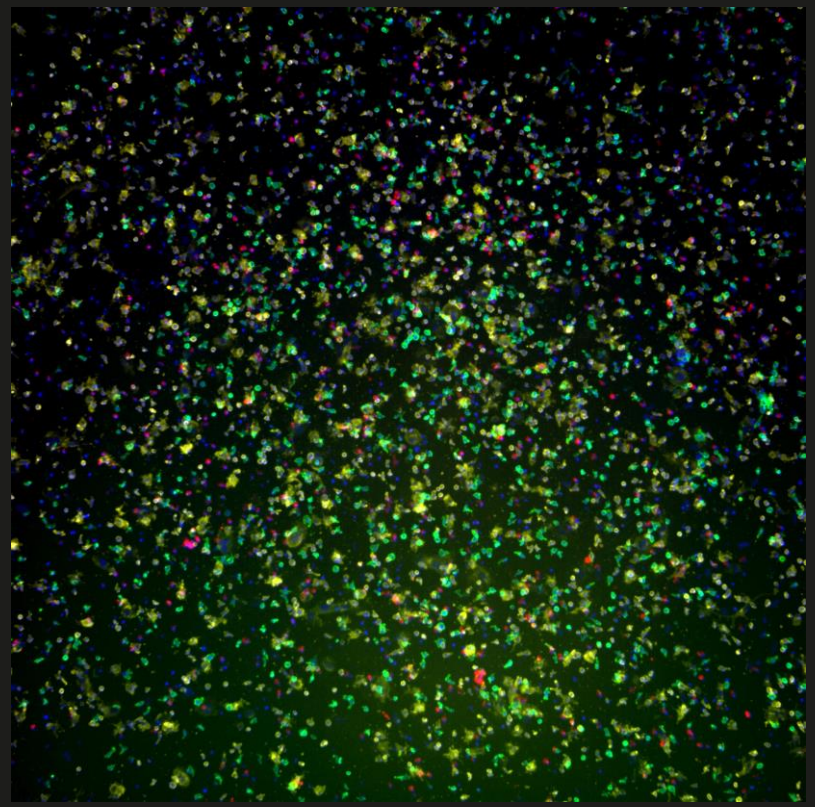
Unstim



SARS



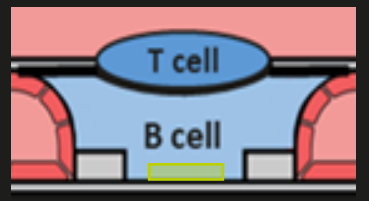
R848



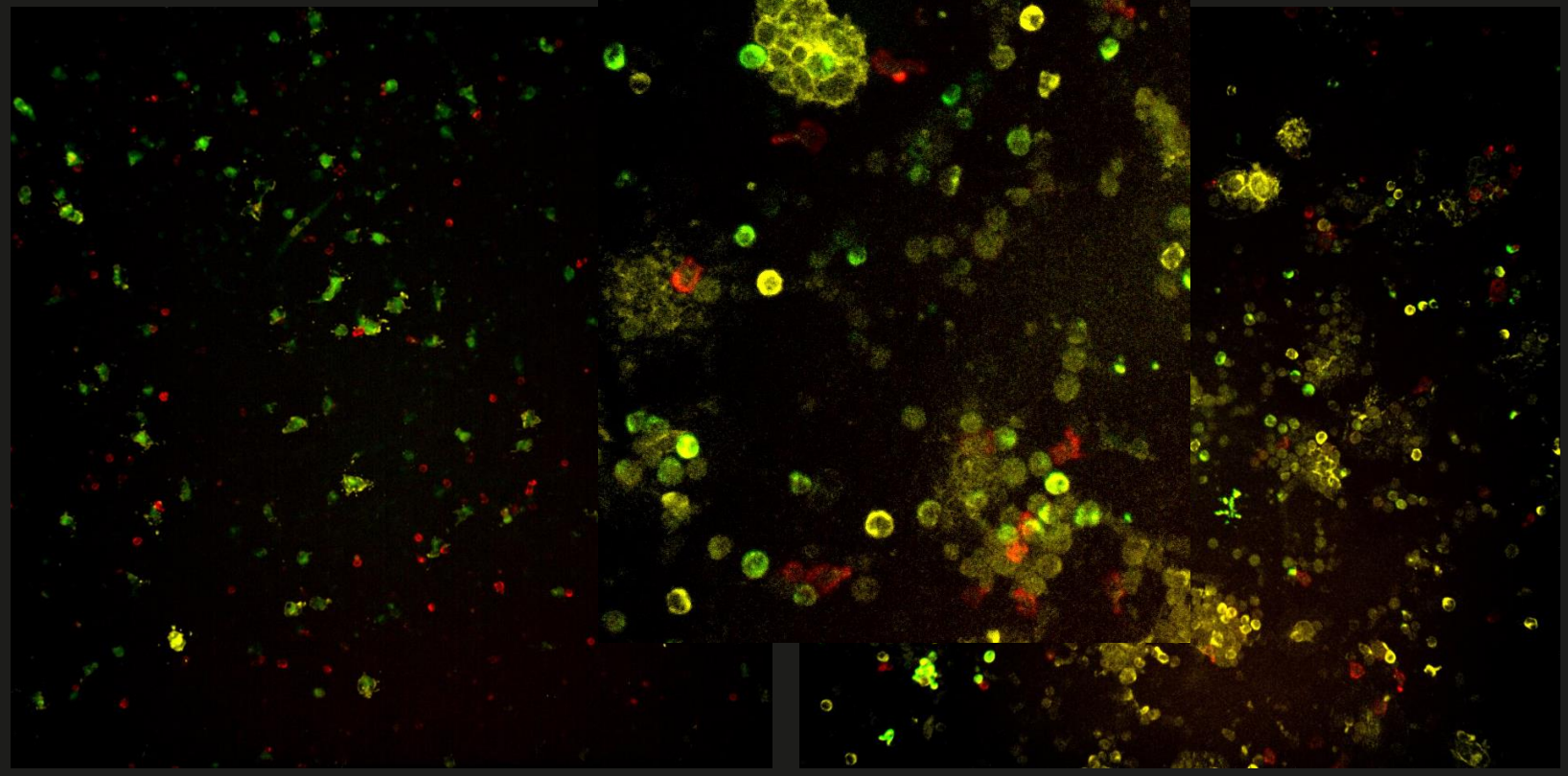
Nuclei CD8 CD4 CD20

MAX projection 10X magnification

IF staining of lymph node chip – post covid

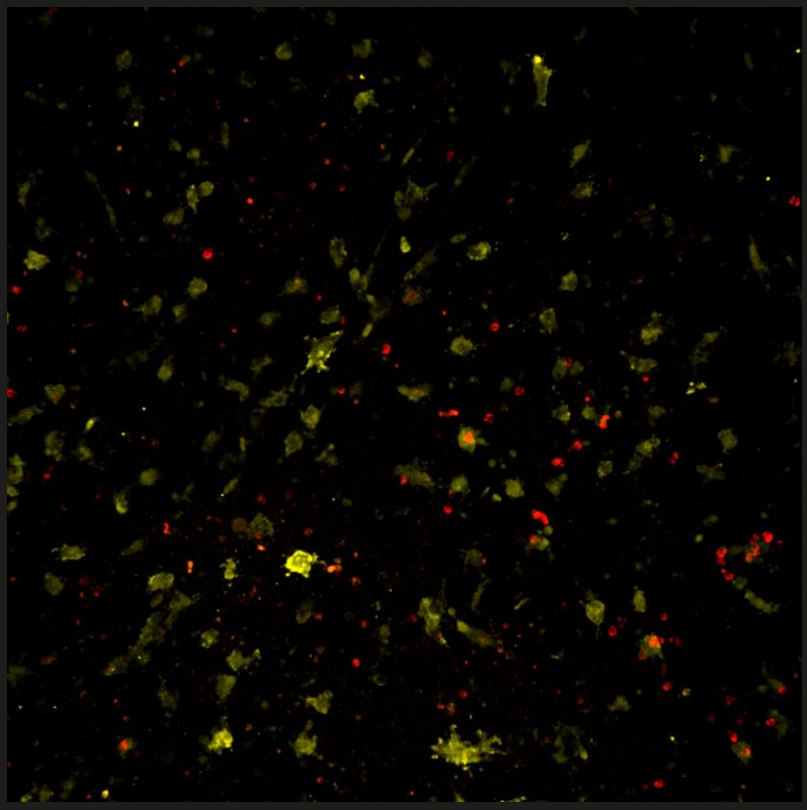


Unstim (MAX 45 Z-steps)



10 Z-steps)

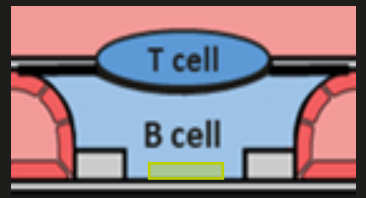
R848 control (MAX 50 Z-steps)

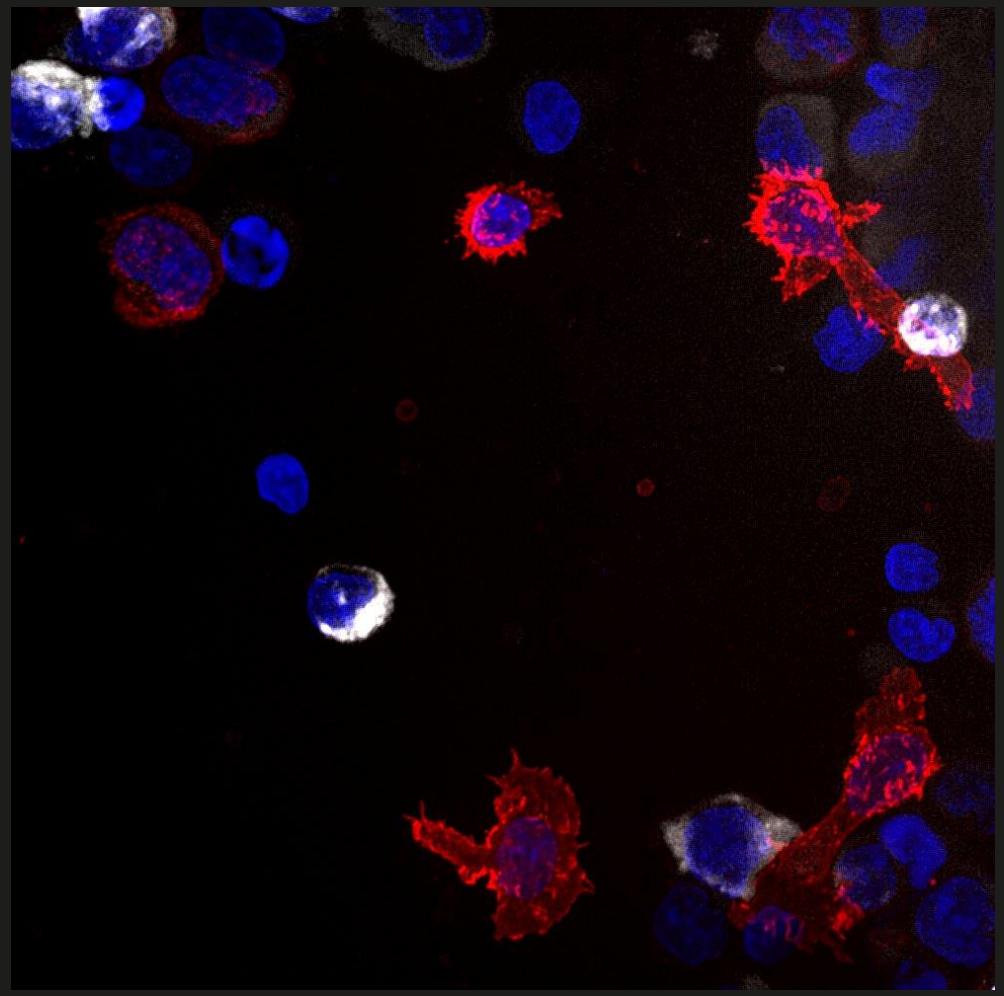


CD4 T cell, CD11c (Dendritic cell), CD20 B cell

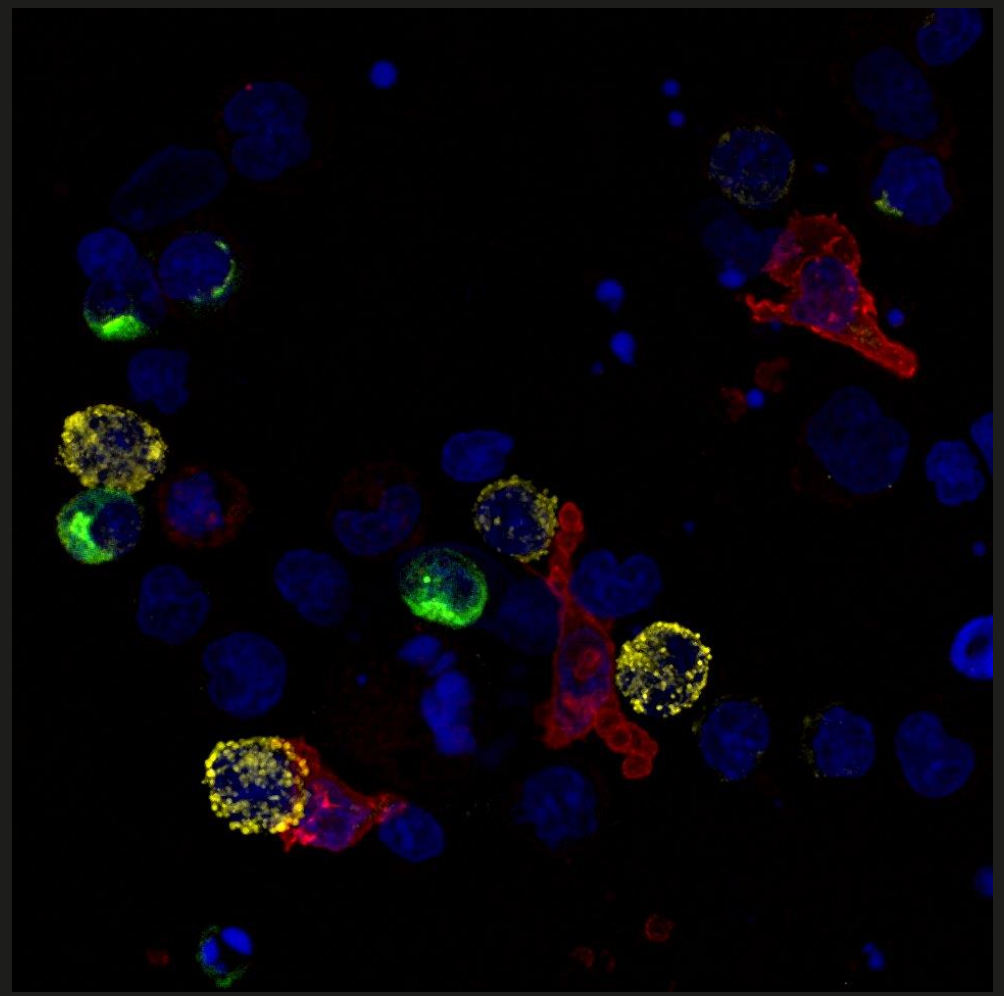
- Dendritic cell and CD4 T cell clustering is observed in SARS-CoV condition.

Interaction of immune cells in SARS-CoV-2 condition





Nuclei, CD19 (B cell), CD4 (T cell)



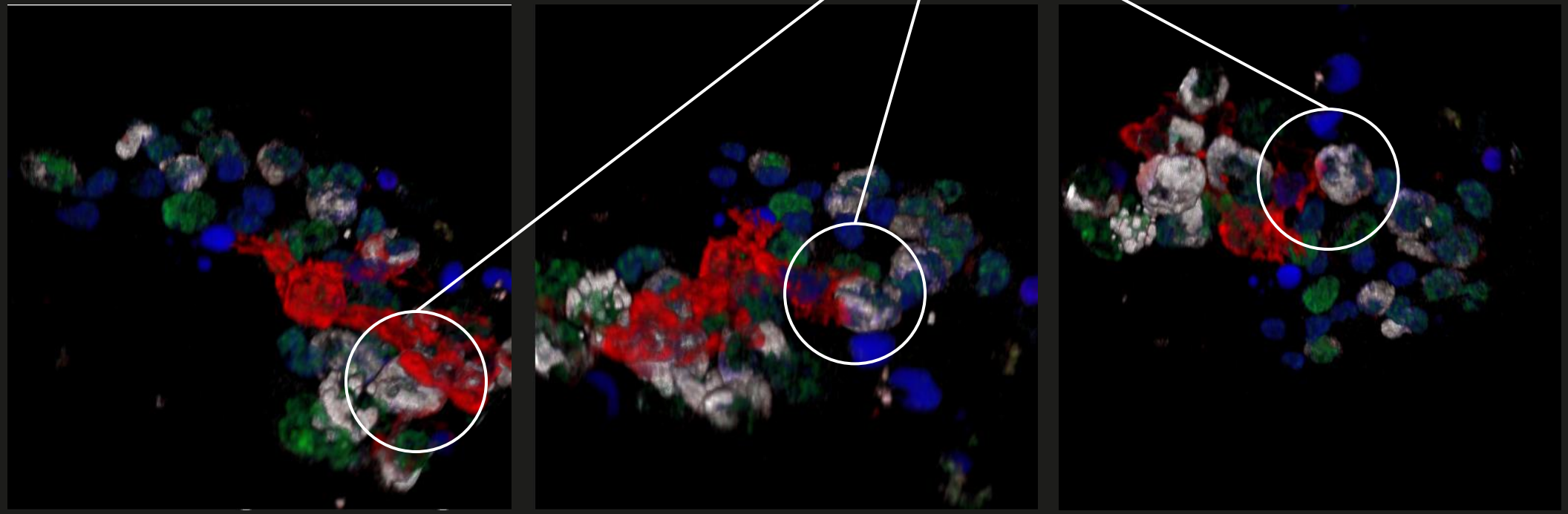
Nuclei, CD4 T cell, CD11c (DC) , CD19 (B cell)

3D reconstruction 60X imaging - post-covid SARS-CoV-2

B cell (red) – T cell (white) interaction

60X magnification (Confocal HtAI)

Nuclei - Hoechst
CD20 – APC
CD4 – AF750



Outcomes in vitro 3D lymph node

- 3D lymph node model can be produced with cryopreserved PBMCs
- Positive control compound (R848) showed consistent responses in the model for flow cytometry (4 out of 4 iterations) and fluorospot (3 out of 4 iterations)
- SARS-CoV exposure did result in consistent responses in the model for flow cytometry (3 out of 4 iterations) and fluorospot (2 out of 4 iterations)
  - Donor differences / timeline
- SARS-CoV-2 exposure resulted in interactions between different immune cells and clustering

CRACK-IT TDAR results highlight the promise of the LN model, though additional refinement is necessary

Immunogenicity and Immunotoxicity

closely interlinked concepts

Immunotoxicity and immunogenicity are closely interlinked concepts, where immunogenicity pertains to the ability of a substance to provoke an immune response, while immunotoxicity refers to adverse effects caused by the immune response to that substance; these interactions can significantly impact therapeutic efficacy and safety.

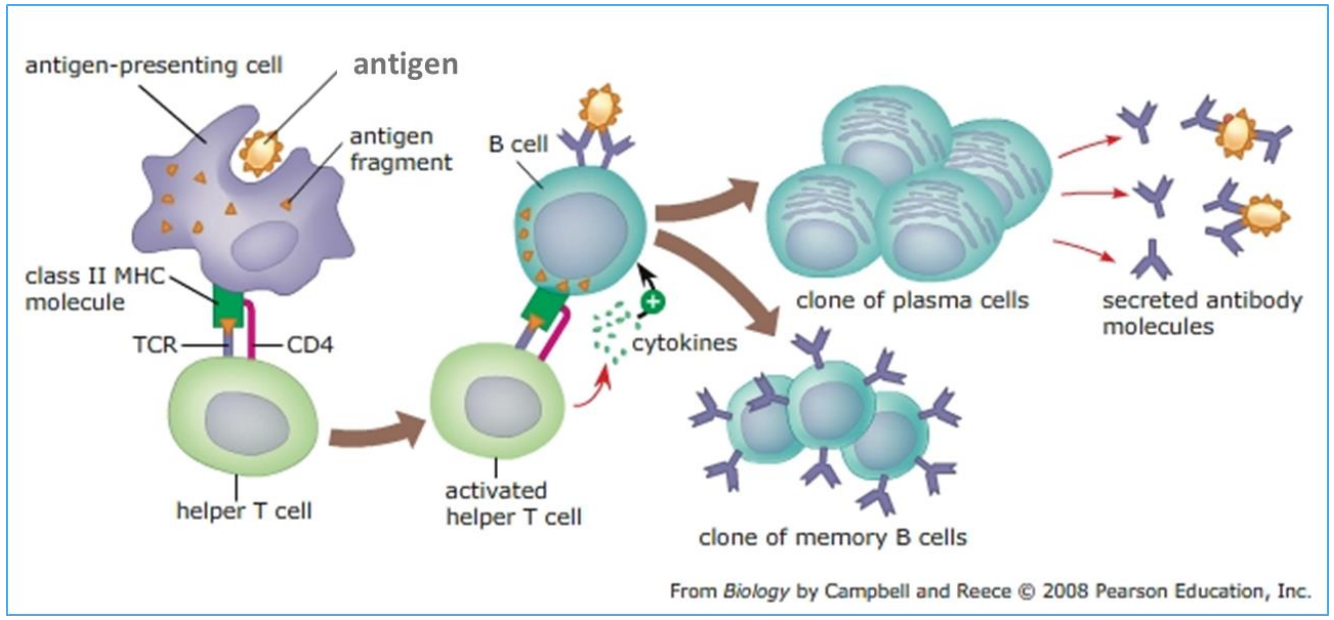
Immunogenicity: This is the capability of a substance (like a drug or vaccine) to evoke an immune response, leading to the production of antibodies or cellular responses against that substance. Immunogenicity can be a desired effect, especially in vaccines, but can also result in undesirable reactions, especially with biotherapeutics that may be seen as foreign by the immune system

Immunotoxicity: This is defined as the adverse effects the immune system experiences due to exposure to foreign substances (immunotoxicants). It can manifest in various forms, including immunosuppression, hypersensitivity, or autoimmune diseases. Importantly, immunotoxicity can be mediated by immunogenic responses leading to unwanted immune system dysfunction.

⇒ **Immunotoxicity** and **immunogenicity** are interrelated in that immunogenic responses to therapeutic agents can lead to both beneficial and adverse effects on health. The interactions of these processes are vital in clinical settings, particularly in drug development, where managing immunogenicity can prevent undesirable immunotoxic effects. Understanding these relationships aids in providing safer therapeutics and advancing treatment efficacy in clinical applications.

Immunogenicity

"The ability of a particular substance, such as an antigen or epitope, to induce an immune response"



WANTED	UNWANTED	
Vaccines	**Therapeutic proteins**	**Cell&Gene Therapy Products**
Immune response against the pathogen (virus, bacteria) aiming at protecting the organism.	Production of antidrug- antibodies (ADAs), possibly neutralising the therapeutic effects of the treatment and, in rare cases, inducing adverse effects.	Cellular and humoral responses Anti-HLA antibodies Immune rejections Potential safety effects

T cell activation/proliferation assays using human PBMC can be used as a surrogate marker for antibody responses: good correlation between T cell activation assays and reported ADA responses (when clinical products are tested in T cell activation/proliferation assays).

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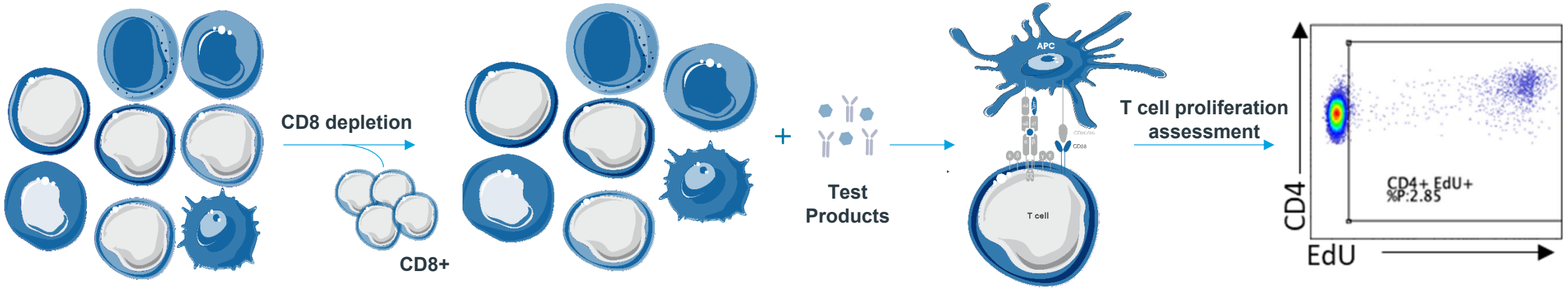
Current approaches : CD8-depleted PBMC assay

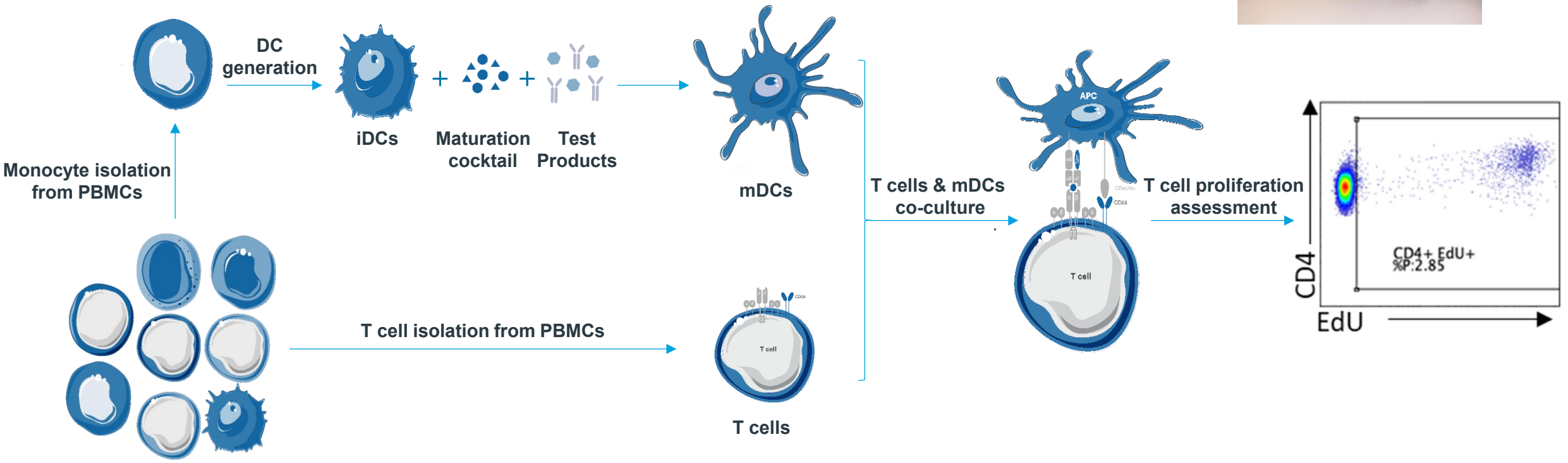


T cell activation and proliferation assays to assess and compare the immunogenicity potential of test molecules

Format depends on the nature and function of the test products:

- The CD8-depleted PBMC format is used for test products with non-immuno-modulatory functions

Current approaches : DC-T cell assay

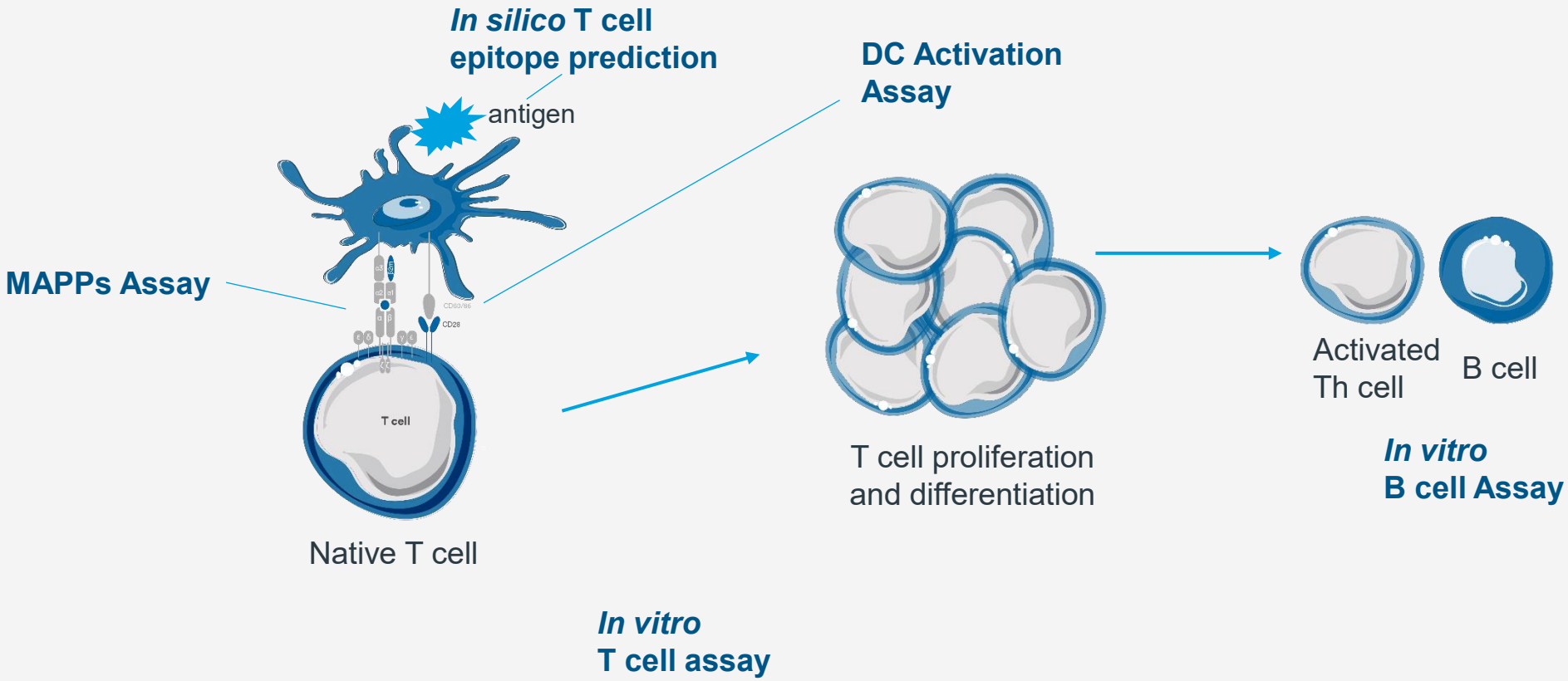


T cell activation and proliferation assays to assess and compare the immunogenicity potential of test molecules

Format depends on the nature and function of the test products:

- DC-T cell format is used for test products with immuno-modulatory functions

Early Immunogenicity Assessment Tools
Current approaches



Benefits Early Immunogenicity Risk Mitigation/Prediction

Assessment/predictive tools have several benefits in the development and design of less-immunogenic drugs and can be used at an early stage to:

+ Improve the safety profile by testing and re-engineering (de-immunization and humanization) or adapted formulations
+ Select the candidates with the lowest immunogenic potential
+ Evaluate the immune responses in different or specific test populations
+ Add an additional quality tag to the pipeline candidates
+ Learn and understand immunological mechanisms of the test products
+ Compare the immunogenic potential of originator and biosimilar candidate

Additional value of the human in vitro T-cell dependent antibody response assay

This is still the missing link to bridge non-clinical with clinical immunogenicity

+ So far, a good understanding in vitro of HLA binding, MHC peptide presentation, DC maturation and T cell responses, **but not yet the following B cell response**
+ Better representation of the whole immune cascade within its full cellular environment
+ Possibility to evaluate impact of T cell epitopes on antibody formation
+ Possibility to evaluate the impact of ADAs without clinical risk in human (neutralizing versus binding antibodies) and identify B cell epitopes

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