

Lymphoid Follicle Microphysiological Systems:

A Platform Technology for Rapid Infectious Disease Countermeasure Development

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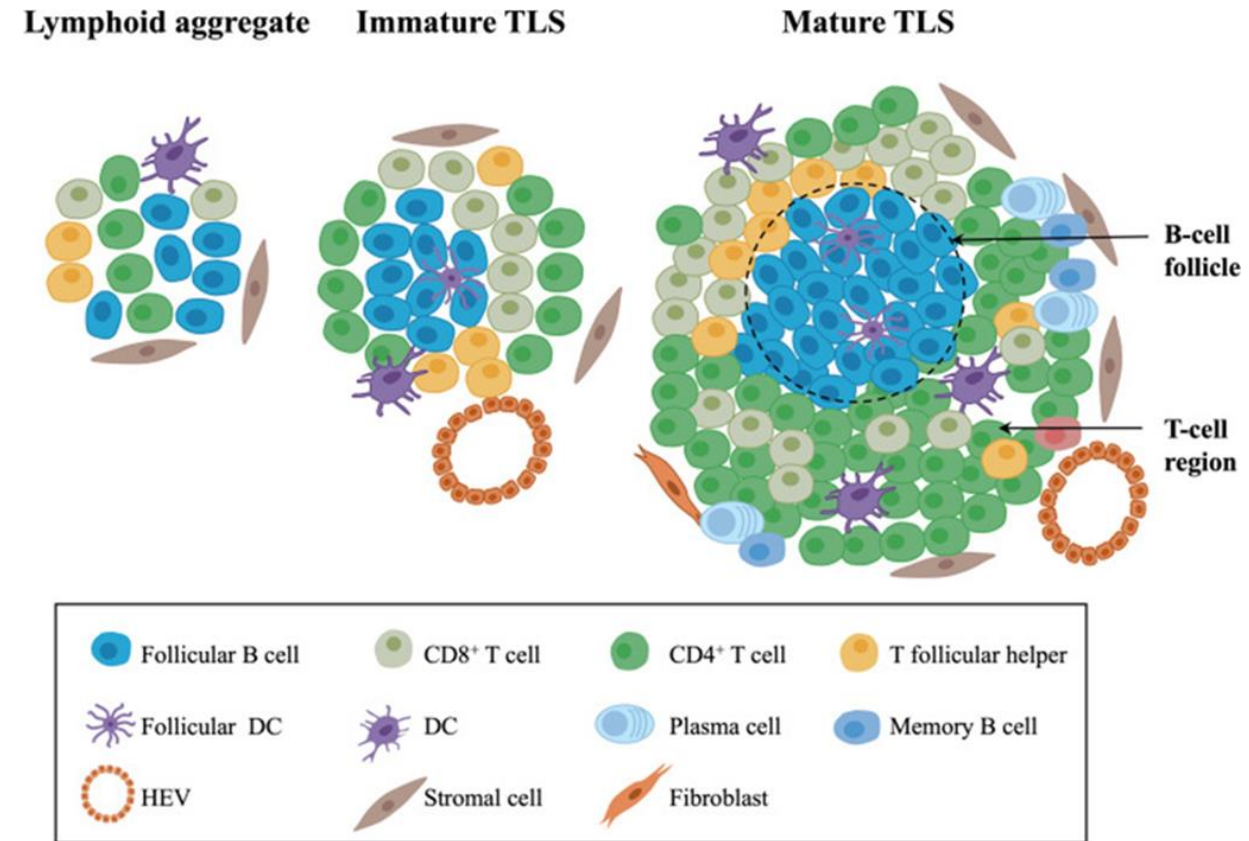
Why Microphysiological Systems?



Microphysiological systems (MPS) offer several advantages in comparison to conventional methods of biological select agent and toxin (BSAT) modeling.

- » 2D cell culture models overly simplistic
- » Animal models do not always reproduce human disease states
 - » Evolutionary divergence of MHC II regions
 - » Absence of HLA I and II
 - » Pathogen displays of human tropism
- » Cheaper and less controversial than animal work

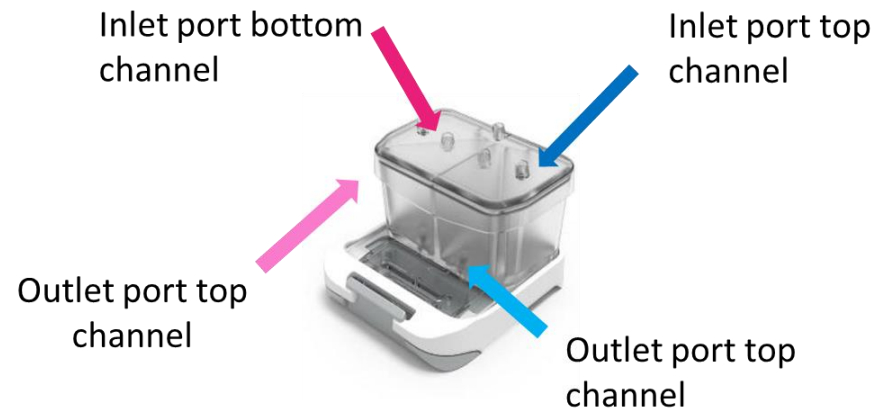
In sum, validating that MPS can accurately reproduce *in vivo* human tissues for producing reliable and translatable scientific findings is of high importance.



<https://pmc.ncbi.nlm.nih.gov/articles/PMC10667730/>

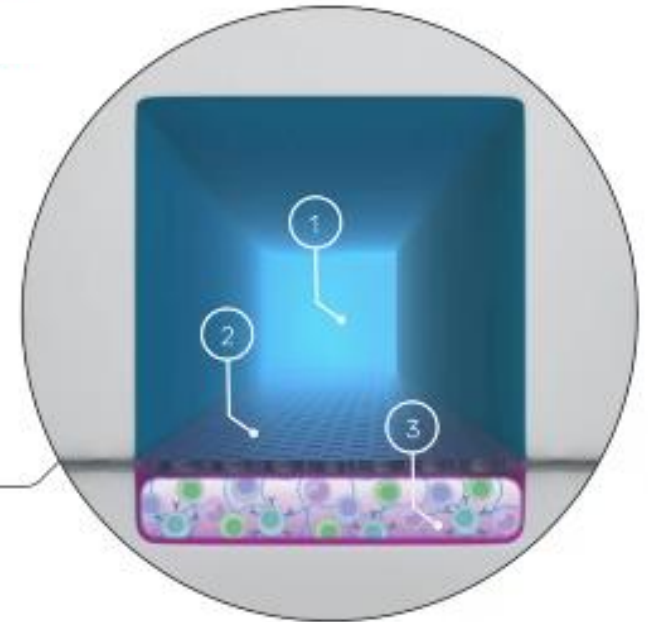


Chip-Pod Interface



LF-Chip Setup

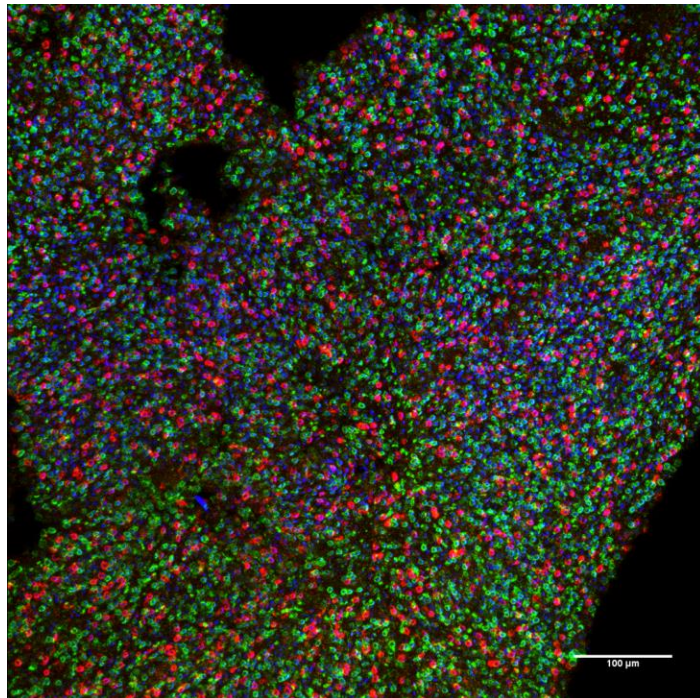
1. Media & Antigen Flow
2. Porous Membrane
3. Immune Cells within ECM



LF-MPS Flow Chip, 4 Days



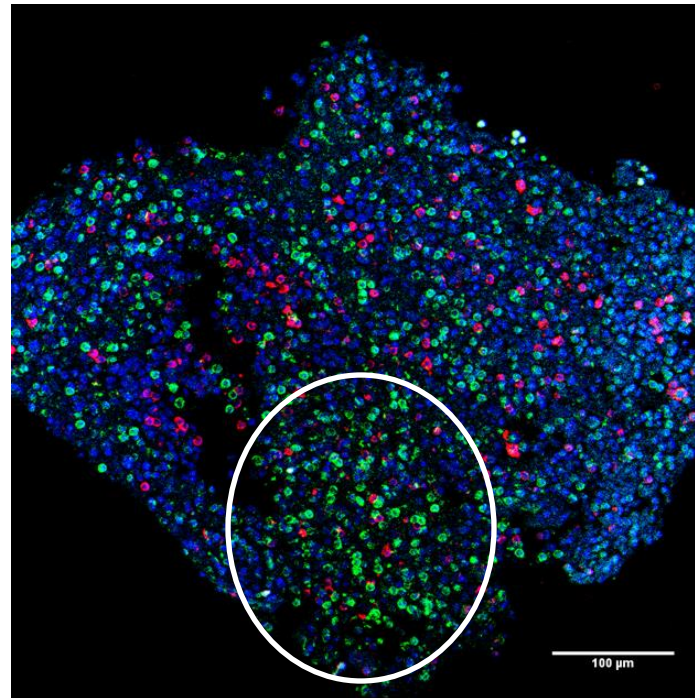
CD20: B cells
CD3: T cells
DAPI: DNA



20X

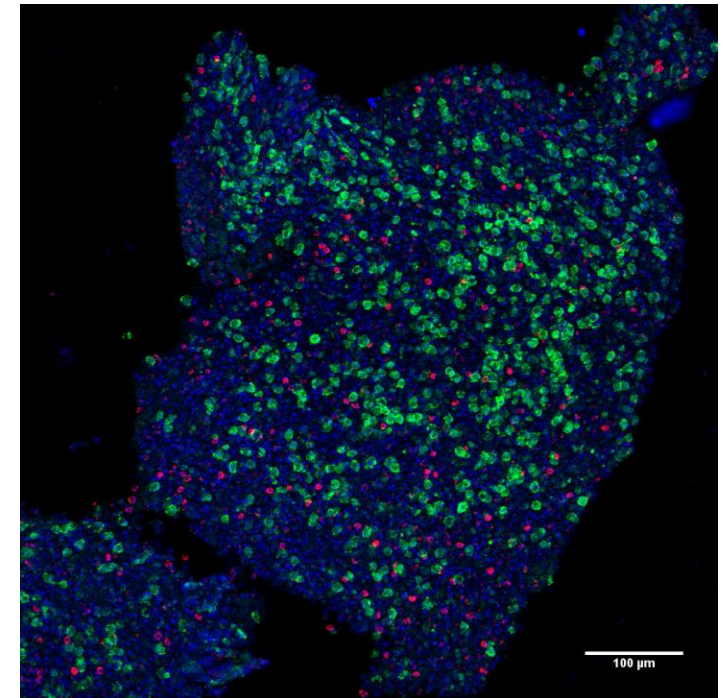
Day 1 Post-seeding

CD20: B cells
CD3: T cells
DAPI: DNA



Day 4 Post-seeding

CD8: Killer T cells
Iba-1: Macrophage
DAPI: DNA



20X

Day 4 Post-seeding



The LF-MPS exhibits a statistically significant, continuous increase in both Tfh and Tfc populations over the 4-day culture period under flow conditions.

T Follicular Helper (Tfh) Cells:

- Provide the signals to drive B-cell maturation, support germinal center formation.

T Follicular Cytotoxic (Tfc) Cells:

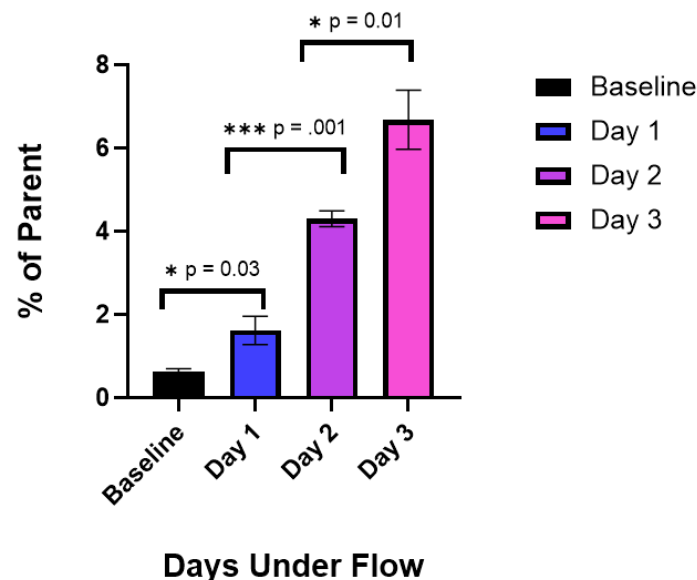
- Parallel increase indicates a capacity to clear infected cells and eliminate autoreactive B cells.

LF-MPS Interpretation:

- Cellular expansion mimics *in vivo* immune kinetics, suggesting the platform's ability to support complex T-B cell cross-talk in the future.

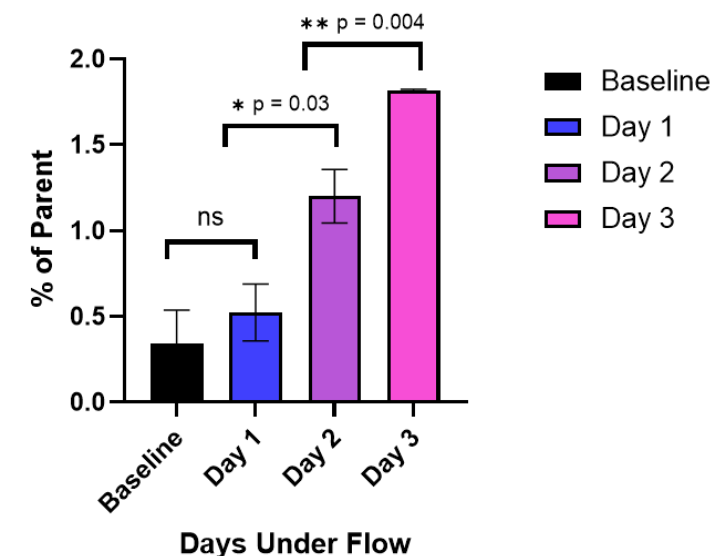
A

Follicular Helper T cells



B

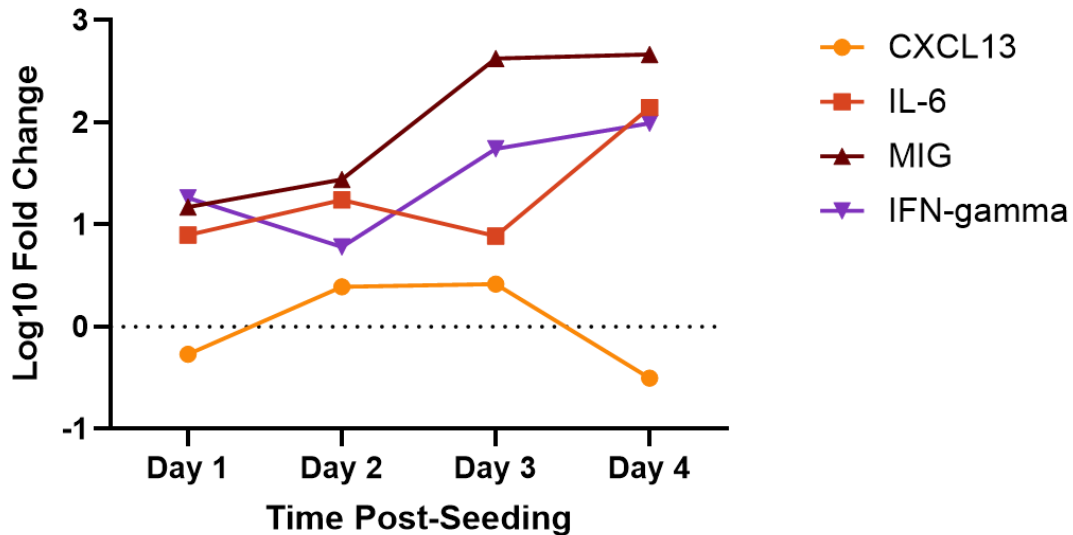
Follicular Cytotoxic T Cells



Flow cytometric analysis of follicular helper T cell differentiation (A) and follicular cytotoxic T cell differentiation (B) over time in flow LF-MPS chips.



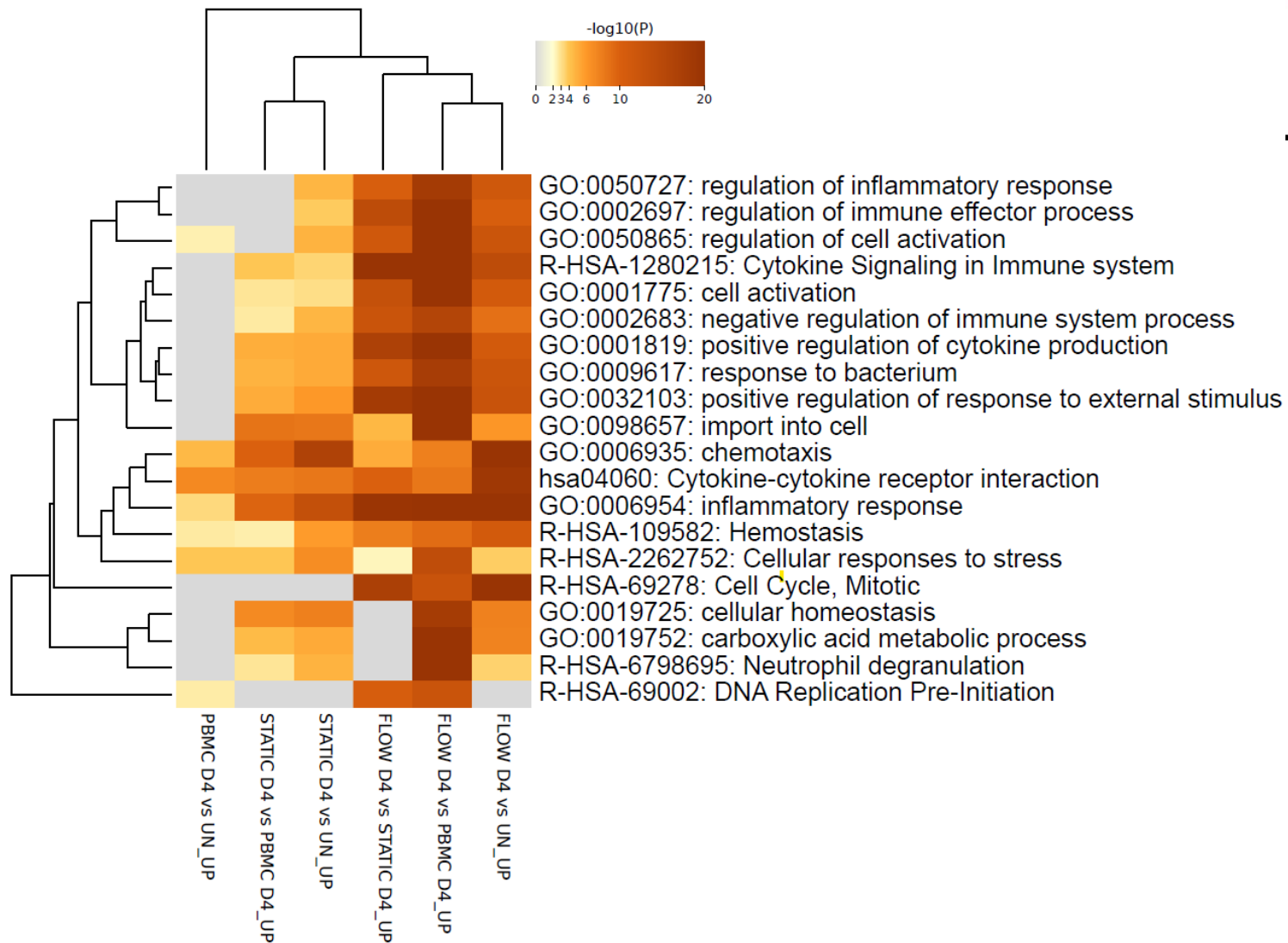
Cytokine Expression



Data synthesized from key cytokine expression indicates LF-MPS recapitulates *in vivo* primary lymphoid follicle development: sequential, cytokine-driven stages of architectural and functional capacities.

Cytokine	Function	Interpretation for LF-MPS
CXCL13	B cell and Tfh cell recruitment, primary lymphoid follicle organization and maintenance ⁶	Transient upregulation: Active B cell recruitment and self-organization of the lymphoid follicle structure. Late downregulation: successful follicle formation is complete; LF-MPS switches to a mature, homeostatic state where the strong organizing signal is no longer required. ⁶
IL-6	B cell survival and differentiation, T cell regulation ¹	Early downregulation: LF-MPS in developmental mode, not inflammatory. Late upregulation: LF-MPS switches to survival and maintenance mode of aggregated cells ¹
IFN-gamma	Pushes immune system towards cell-mediated response ^{2,3}	Appears late, produced by nascent T cell zone for further organization and macrophage priming ^{2,3}
MIG	T lymphocyte recruitment ⁵	Early downregulation: B cell recruitment prioritized Late upregulation: T cell recruitment for T cell zone formation ⁵

Transcriptomic Analysis: Day 4 Flow, Static, and 2D PBMC Cultures, Upregulated Pathways



Top 20 upregulated pathways: Molecular and Cellular Reprogramming

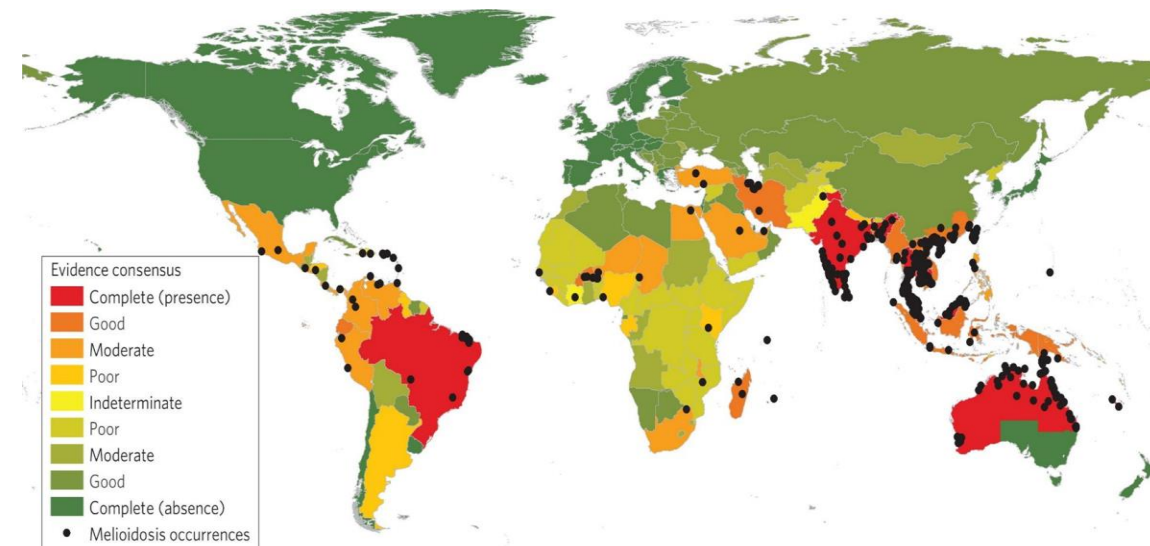
- Mechanosensitive Pathway Activation
- Functional Signaling Shifts
- Dynamic Chemotactic Guidance
- Phenotypic Transition

What is *Burkholderia pseudomallei*?



- Soil-dwelling pathogen endemic to INDOPACOM, particularly southeast Asia and Northern Australia
- Particularly useful pathogen to study infection dynamics:
 - Bp is aggressive and facultatively intracellular
 - Bp-host immune system engagement been reasonably well-characterized
 - Bp infection induces unique changes to the host immune response not seen during infection by other bacterial pathogens.
- Exempt strain JW270 used in these studies
 - Missing capsule gene, retains all other virulence factors

Global Distribution of *Burkholderia pseudomallei*

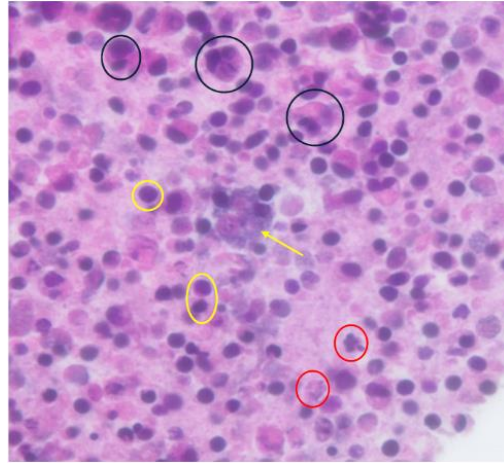
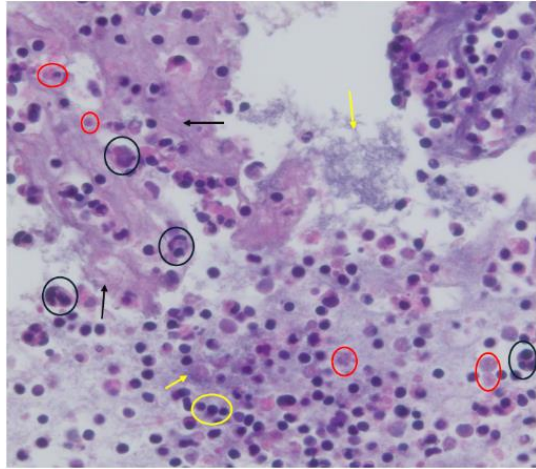


<https://www.nature.com/articles/nmicrobiol20158>

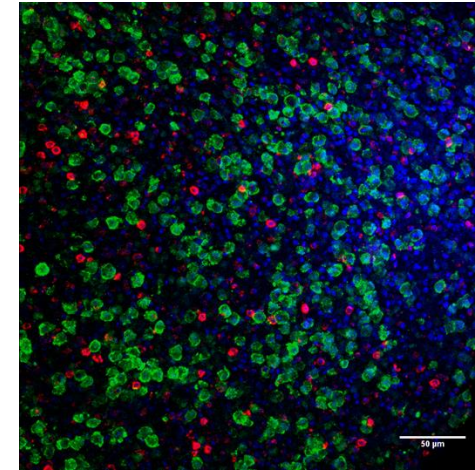
H&E Staining; Flow Conditions; Infected w/ JW270 MOI 10



H&E, 60x

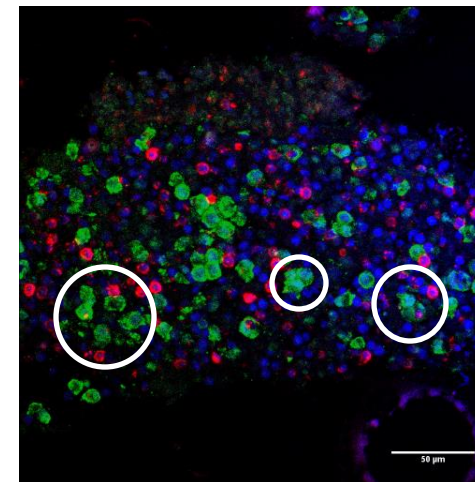


- = Small lymphocytes
- = Phagocytic cells
- = Degenerating/necrotic cells
- ↓ = Amphiphilic matrix
- ↓ = Bacteria

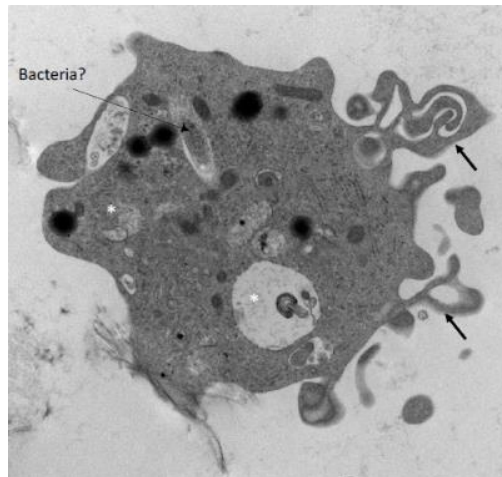
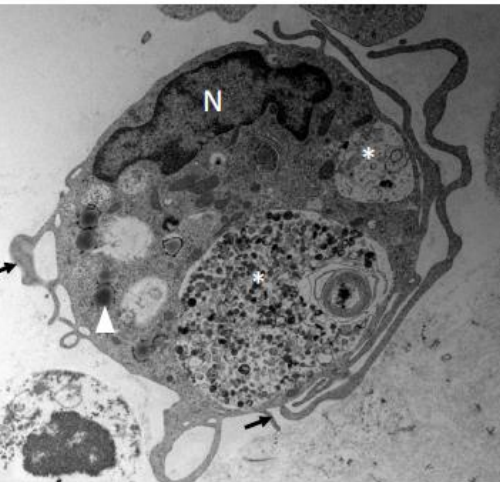


Uninfected

CD8: Killer T cells
Iba-1: Macrophage
DAPI: DNA



Infected, MOI 10



* = Autophagocytic cytoplasmic structures in various stages of degradation

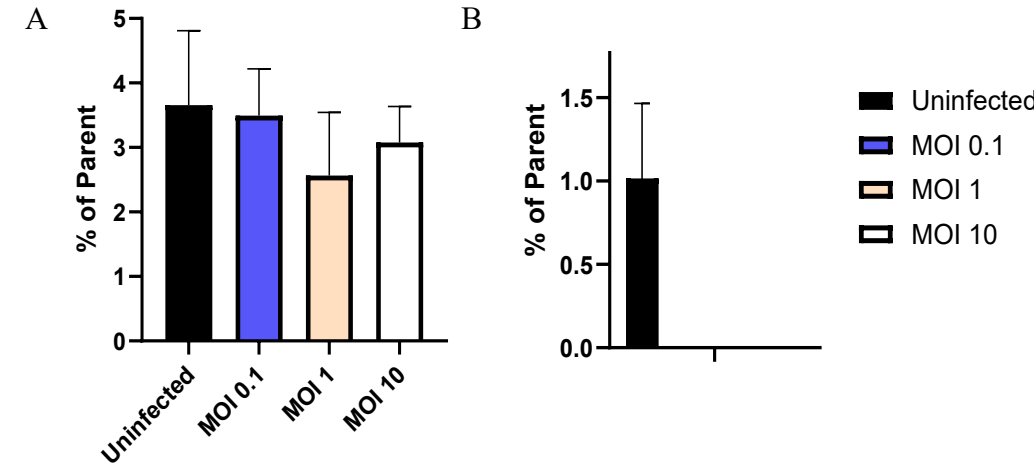
N = nucleus
M = mitochondria

↓ = Surface projections

Lymphoid Follicle Microphysiological System

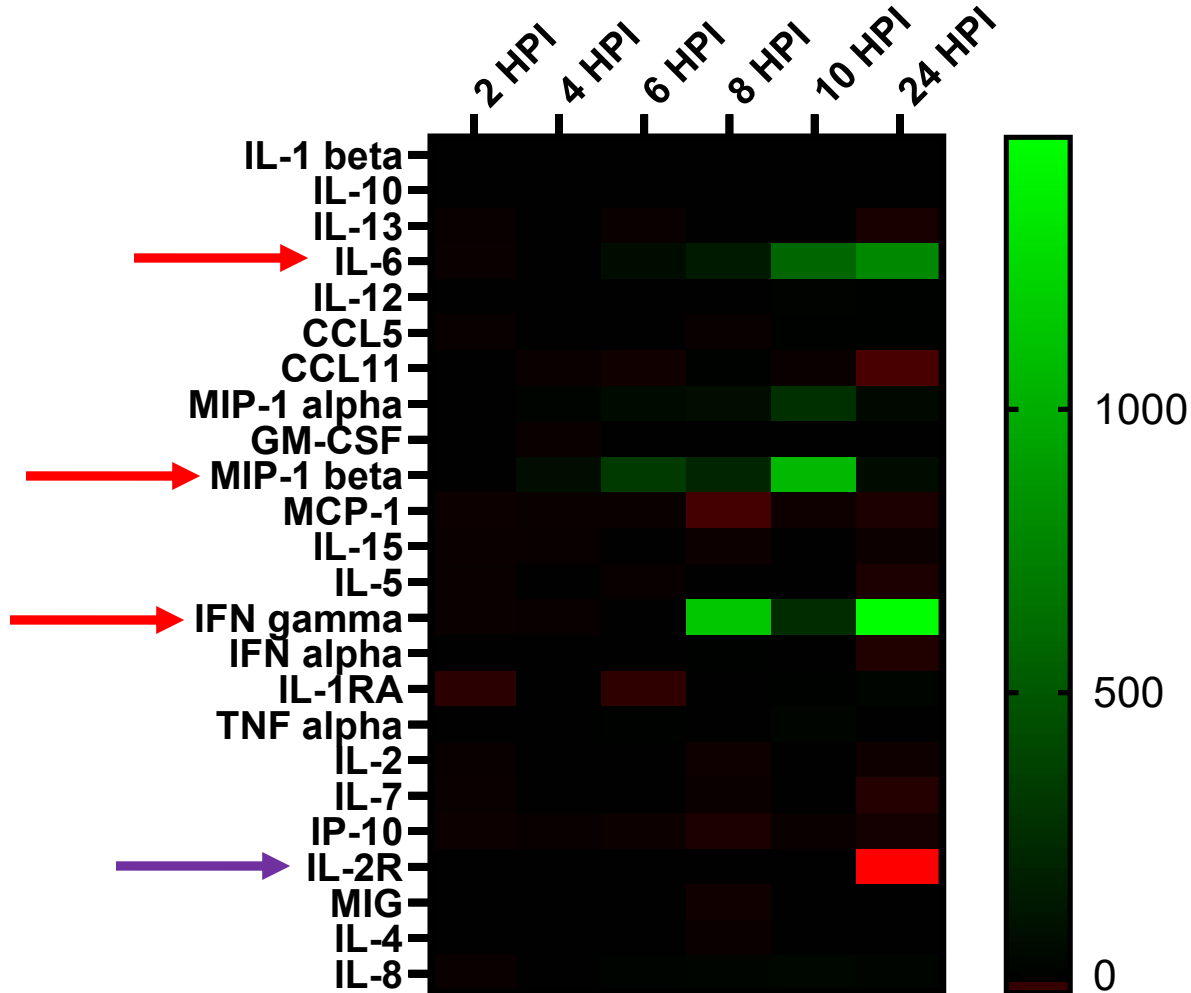


Cell Type	Parent Population	Subpopulation	Uninfected	MOI 0.1	MOI 1	MOI 10
PBMCs	Live singlets	-	69.57 ± 2.56	70.6 ± 1.47	72.64 ± 1.32	72.32 ± 1.8
T Cells (CD3+)		-	64.00 ± 1.93	64.09 ± 1.98	65.5 ± 0.86	67.31 ± 1.15
	Cytotoxic T CD8+	-	35.82 ± 1.06	35.31 ± 0.42	35.14 ± 0.70	35.94 ± 1.21
-	-	CD8+PD1+	1.32 ± 0.51	1.92 ± 0.37	2.05 ± 0.52	2.47 ± 0.83
-	Helper T CD4+	-	51.81 ± 0.62	50.82 ± 0.30	50.83 ± 1.13	52.16 ± 1.07
-	-	CD4+PD1+	3.65 ± 1.15	3.49 ± 0.72	2.56 ± 0.98	3.07 ± 0.56
-	-	CD4+CXCR5+	3.28 ± 0.11	3.49 ± 0.41	3.48 ± 0.47	3.74 ± 0.68
Non-T (CD3-)	Myeloid (CD20-)	-	69.57 ± 2.56	70.68 ± 1.47	70.91 ± 1.09	72.32 ± 1.87
-	-	Macrophage (CD11b-CD64+)	4.96 ± 0.21	5.90 ± 0.50	8.42 ± 1.26	6.92 ± 0.27
-	-	Monocyte (CD11b-CD34+)	10.55 ± 5.27	5.13 ± 1.08	4.51 ± 4.25	1.05 ± 0.69
-	-	FDC (CD163-CD35+)	1.27 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
-	-	M2 (CD163+CD35-)	7.48 ± 9.93	2.47 ± 4.27	0.00 ± 0.00	0.00 ± 0.00
-	-	NK (CD163-CD35-)	69.29 ± 16.7	73.8 ± 3.41	63.78 ± 19.6	100 ± 0.00



Flow cytometric analysis of CD4+PD1+ (A) and follicular dendritic-like cells (B) 24 hours post-infection (HPI) from MOIs 0.1, 1, or 10.

Cytokine Fold Changes, MOI 10



****Note:** IL-17A was expressed 200,000 times higher; inclusion in heat map threw off results

Cytokine	Clinical Bp infection
IL-17A	Associated with increased sepsis and melioidosis presentation ¹
IL-6	Association of increased IL-6 with worsened disease severity ²
IFN-gamma	Increased IFN-gamma production correlated to severe melioidosis ¹
MIP-1 beta	Treatment with MIP-1 beta inhibitors may improve melioidosis outcomes ³

qPCR analysis, 24 hours post-infection



6 Days Post-Seeding Uninfected vs Burk MOI 0.01		
	Gene	Fold Change
Upregulated	NOS2	543.07
	IL1A	42.32
	IL7	15.52
	CCR2	13.24
	IL1B	9.99
	CSF2	6.85
	C3	5.94
	CD34	4.65
	CSF3	4.59
	LRP2	4.59
Downregulated	FN1	69.53
	IL17A	32.99
	IL9	16.15
	AGTR2	11.59
	SELE	11.20
	AGTR1	6.24
	CYP1A2	4.18
	CD8A	3.32
	HLA	2.80
	CXCL10	2.78

Gene	Function	Interpretation for LF-MPS
IL-17A	Dysregulation is a hallmark of acute, severe Bp infection ¹	LF-MPS reproduces a known Bp infection marker
NOS2/CCR2/CSF3/C3	Innate immune responses	LF-MPS producing innate immune responses
SELE	E-selection, endothelial activation marker, vascular dysfunction ^{2,3}	LF-MPS reproduces a Bp infection transcriptomic signature found <i>in vivo</i>
CD8a/HLA	T cell gene expression disruption	LF-MPS adaptive immune response

-  = Innate immune response
-  = Bp-specific response
-  = Matches *in vivo* mouse data
-  = Adaptive immune system

Preliminary New Findings in Bp Pathogenesis



Observation	Mechanism	Consequence
FDCs obliteration	Antigen presentation scaffold destroyed	B cell response paralyzed
CD8a gene downregulation	Cytotoxic T cells stop expressing CD8	CD8+ cells cannot identify/kill infected cells
HLA gene downregulation	Antigen presentation suppressed	Avoid T cell recognition
Downregulation of IL-2R 24 HPI	T cells cannot respond to IL-2	Failure to mount strong T cell response



Validated the Lymphoid Follicle Microphysiological System (LF-MPS)

- Successfully promotes spontaneous immune cell organization & differentiation (T_{fh}, T_{fc}); produces key cytokines needed for primary lymphoid follicle formation

Modeled Clinically Relevant Infection

- Supports bacterial invasion, replication within host cells, and key host responses including *Burkholderia*-specific cytokine and transcriptomic dysregulation

Enabled Novel Discoveries

- Establishes a foundational tool for investigating acute-phase diagnostic biomarkers and discovering new mechanisms of pathogenesis

Future Directions:

- Transcriptomic benchmarking: Perform RNAseq on *Burkholderia*-infected chips to compare with in vivo data
- Strain comparison
- Pathogen expansion



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QUESTIONS?



Our Mission & Vision



Mission

Responsively and responsibly create, develop, acquire and deliver medical capabilities for the warfighter.

Vision

Lead the advancement of military medicine.

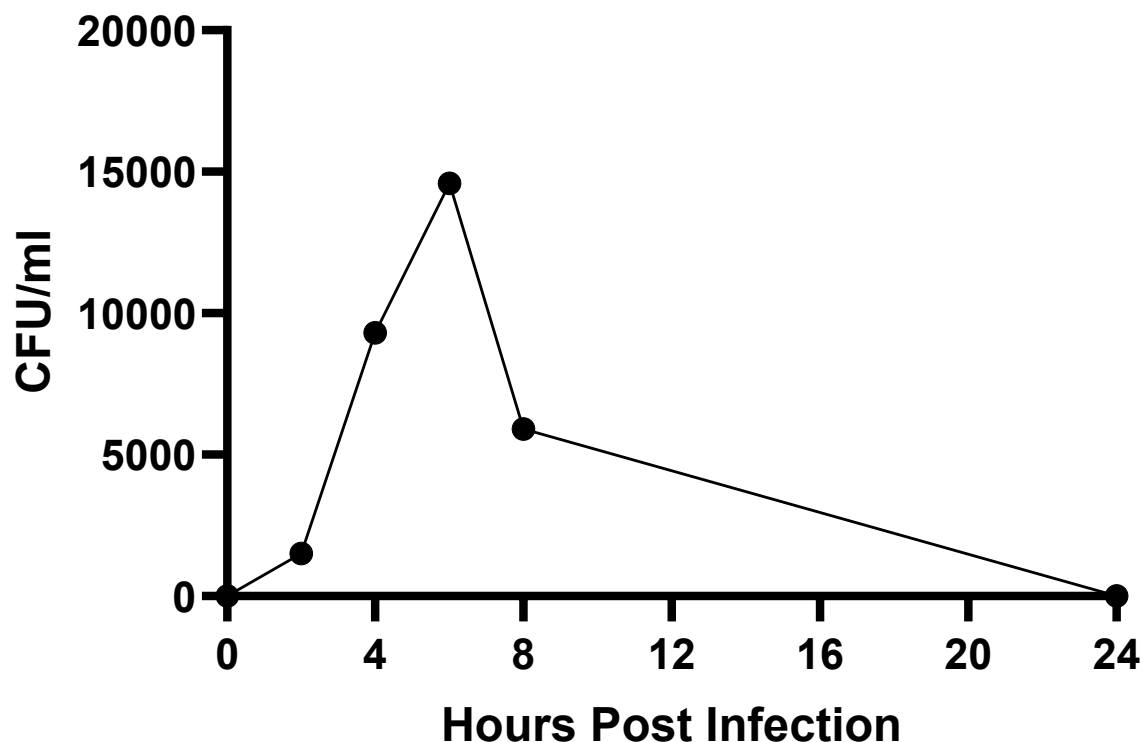


“Military medicine is not just about treating injuries; it’s about ensuring the health and readiness of those who serve, so they can accomplish their mission and return home safely.”

- MG Paula C. Lodi, Commanding General, USAMRDC



Bacteria CFU/ml vs Hours Post-infection



- CFU counts from an exploratory time course experiment.
- Purpose of this experiment was to determine the extent to which bacteria were invading and multiplying inside the PBMCs present in the LF-MPS.
- Bacteria invade and replicate in the cells of this model, with 6 hours post-infection (HPI) producing the highest intracellular bacterial counts.