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An Immunologic Evaluation of T cell Dynamics and Immune Subsets in Aged Mice

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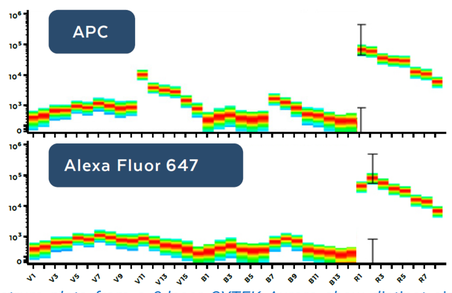
Background

Immunosenescence, the age-related decline in immune function, contributes to disease susceptibility. At NUVISAN ICB, we utilise advanced spectrum cytometry which enables comprehensive analysis of immune cell markers to reveal complexities in aging of cellular composition, activation, and function. This technique identifies T cells, B cells, natural killer cells, and myeloid subsets involved in aging and assesses markers linked to senescence and exhaustion. It can uncover cytokine shifts, receptor expressions, and signalling changes, clarifying compromised responses in the elderly and aged animals. Coupled with cytokine levels and behavioural testing in aged mice, spectrum cytometry enhances understanding of immune aging.

Here, we investigated T cell production in aged mice impacted by thymic involution using spectrum cytometry. Correlations between T cell changes and assessing cytokine levels provided insights into the complexity that may influence immunosenescence. By illuminating potential alteration in cognitive abilities of aged individuals, we aimed to integrate behavioural testing with the T cell dynamics observed in spectrum cytometry. In summary, spectrum cytometry combined with cytokine analysis and behavioural testing in aged mice revealed intricate immune cell dynamics, phenotypic changes, and functional adaptations. This integrated approach may be used for further analyses to deepen comprehension of immunosenescence, aiding targeted interventions for improved immune health in human aging.

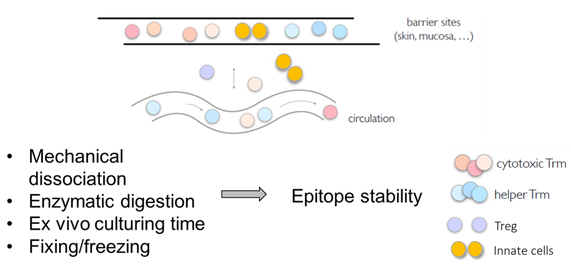
Employing Spectral Cytometry

A



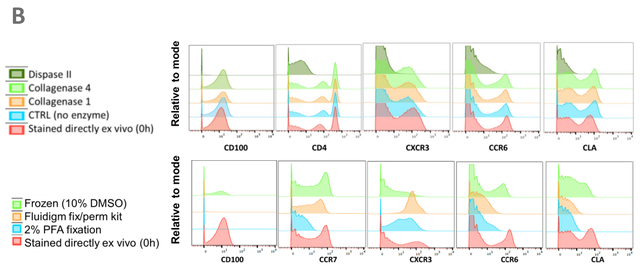
A) Full spectrum plots from a 3-laser CYTEK Aurora show distinct signatures for APC and Alexa Fluor 647.

C



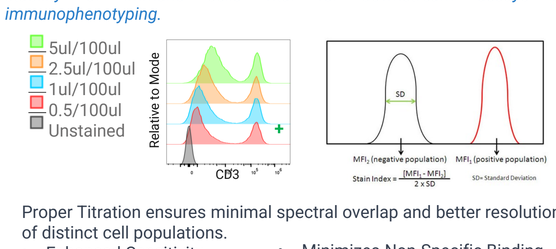
C) Establishment of multiparameter panels require proper assessment of isolation and preservation protocols as practical considerations on epitope stability of immune cells vital for downstream assays and immunophenotyping.

B



B) Representative examples of effect of collagenase 1, 4 and Dispase II after 6 hours of digestion, 37 °C onto epitope recovery. Representative examples of effect of fixation and freezing onto epitope recovery. Human PBMCs, gated on live single CD45+ cells

D



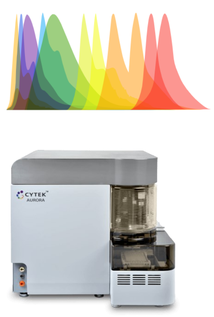
Proper Titration ensures minimal spectral overlap and better resolution of distinct cell populations.

- Enhanced Sensitivity
- Specificity Improvement
- Cost-Efficiency
- Reproducible & Saves Time
- Minimizes Non-Specific Binding
- Reduces Sample Variability
- Maintains Cell Viability
- Facilitates Multicolor Panel Design

D) Representative example of CD3 titration for the identification of the best stain index value .

Off the shelf NUVISAN panels available

Cytek Aurora (VBR lasers)



Murine Immunophenotyping Panel (≥ 28 parameters) of B, T, NK, DC, and monocyte subsets in thymus, blood, spleen, BM, tumors.

Human Immunophenotyping Panel (≥30 parameters) of B, T, NK, DC, ILC and monocyte subsets in PBMCs.

Rat Immunophenotyping Panel (≥ 13 parameters) of B, T, NK, DC, and monocyte subsets in thymus, blood, spleen, BM, tumors.

mouse	human	rat
CD4	CD11c	CD4
CD19	CD45RA	CD45RA
ly6G	CD3	CD8
CD69	CD25	CD3
CD45	IgD	CD62L
CD44	CD95	Gr
CD11b	CD11b	Ki67
CD62L	CD38	CD161a
NKp46	CD57	CD45RC
CD137/41bb	CD27	Live/dead
CD8	CD123	CD28
CD45R/B220	CD127	IgM
CTLA4	HLADR	CD38
F4/80	CCR7	CD45
CD3	CD19	
Ly6c	CD16	
TCRgd	TCRgd	
CD11c	CD14	
PD1	CD8	
CD25	CD1c	
SLAMF7	PD1	
CD206	CD56	
Live/dead	CD45RA	
MHCII(IA/IE)	CD28	
CD38	SLAMF7	
	CXCR3	
	CCR6	
	cKIT	
	IgM	
	Live dead	

Advantage of Spectral flow cytometry:
Less sample material needed to extract more complex information!

Establishments & optimisations of FACS panels are crucial steps in flow cytometry experiments, as they optimize sensitivity, specificity, and reproducibility, leading to improved data quality and reliability. Find out more at https://www.nuvisan.com/home.html to see how we are committed to supporting cutting-edge research endeavours. As part of our comprehensive suite of services, we take great pride in offering specialized assistance as well as expertise in immunology and Fluorescence-Activated Cell Sorting (FACS) experiments. **SCAN here →**

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Murine immunophenotyping

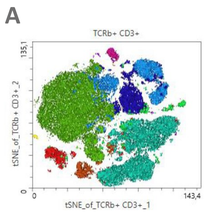
Experimental design:

Randomization. i.p. injection of saline and growth hormone; 3x per week for 13 weeks

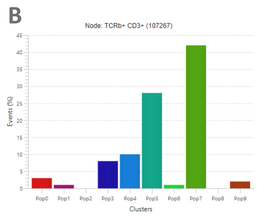
Behavioral testing

Mice were euthanized after 13 weeks of treatment. Blood leukocytes were isolated and analyzed on CYTEK using 20 surface markers.

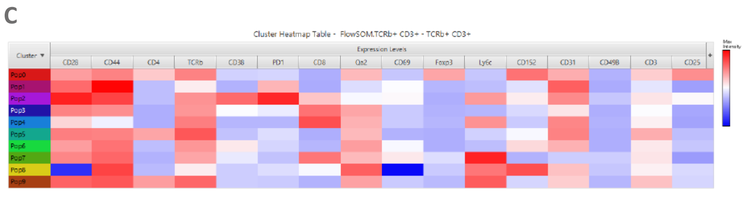
A



B

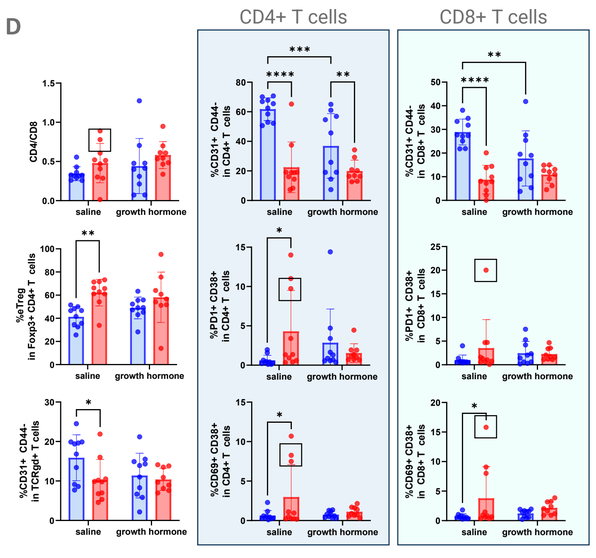


C

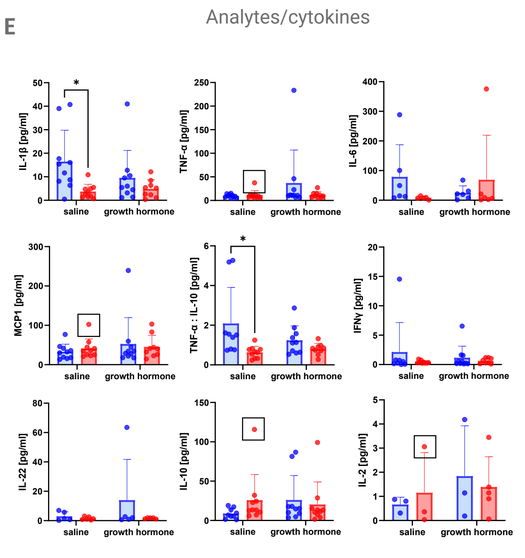


*A) Identification of differentially distributed cellular phenotypes by opt-SNE Probability Binning. Representative example of opt-SNE from freshly isolated blood from a 21 months old mouse showing TCRab+ CD3+ cell subsets. **B & C)** TCRab+ CD3+ populations in murine blood identified and visualised by FlowSOM. **C)** . The heatmap shows median marker intensities within each immune population (red represents high and blue represents low intensity).*

D



E

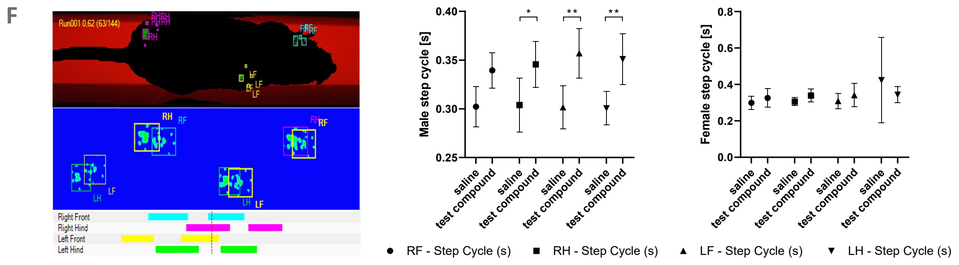


Male
Female

*D) Selected populations of T cells. Populations of T cells were gated according to established lineage markers in all mice (n = 10). **E)** Cytokine analysis. Concentrations of pro- and anti-inflammatory cytokines were determined (n = 10). Blue and red depict male and female mice, respectively. Bars indicate mean ± SD, *P < 0.05, **P < 0.01 ***P < 0.001 , ****P < 0.0001 2-way ANOVA test. Squares in **E and D** demonstrate a representative individual that showed elevated cytokine levels as well as increased frequencies of activated/effector T-cells compared to other individuals from same group.*

Conclusion: Spectrum cytometry showed gender specific alterations in the populations of naïve CD4+ and CD8+ cells in the saline control group with higher frequencies of both cell populations in males. Upon treatment, the frequency of cells in both subsets were observed to decline in males. For individual mice, direct correlations between increased frequencies of effector T cell subsets and elevated cytokine levels could be made. In summary, spectrum cytometry assessing T cell subsets combined with cytokine analyses revealed not only the complexity of immune cell dynamics in aged mice but also highlighted the necessity to carefully consider inter-individual variations.

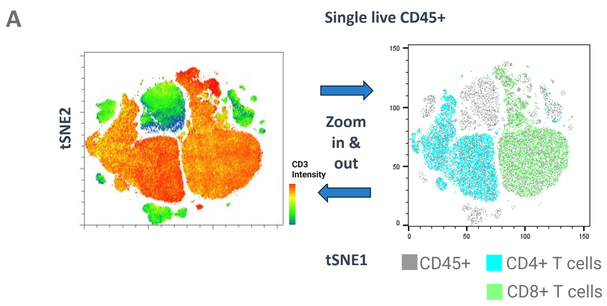
F



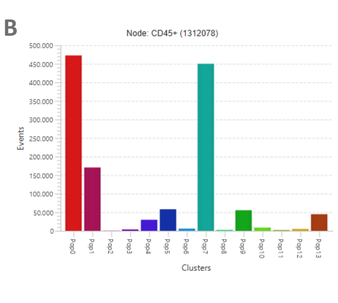
*F) Catwalk analyses of 21 months old male and female mice. Gait analyses revealed gender specific alteration in step cycle duration after treatment (n = 10). Boxes indicate mean ± SD, *P < 0.05, **P < 0.01 2-way ANOVA test.*

Perspective: From Mouse to Human

A



B



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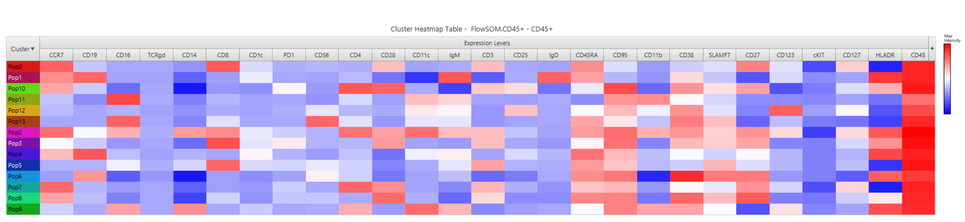
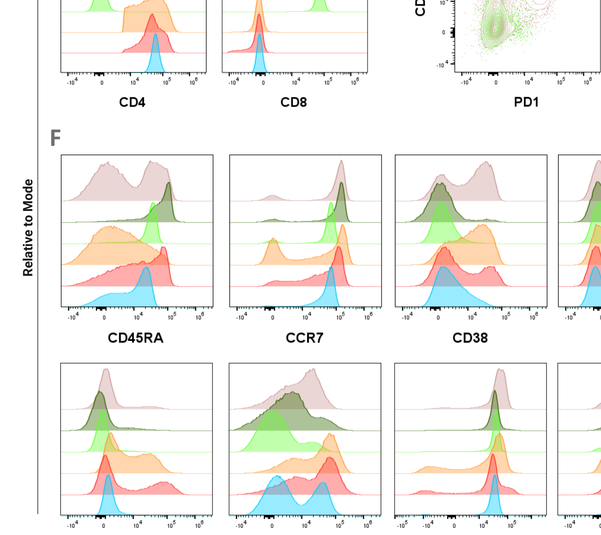


Naïve T cells
CCR7+
CD45RA+
CD27+
CD28+

Antigen exp T cells
CD45RA-
CD27+-
CD28+-

Antigen exp T cells
CD45RA+
CD27-
CD28-
PD1+

D



E

CD8+ T cells/ stimulated
CD8+ T cells CTRL
CD8+ T cells /ex vivo (0h)
CD4+ T cells/ stimulated
CD4+ T cells CTRL
CD4+ T cells /ex vivo (0h)

F

*A) Identification of differentially distributed cellular phenotypes by opt-SNE Probability Binning. Representative example of opt-SNE from freshly isolated PBMCs from 1 healthy donor showing CD3 intensity among cell subsets. **B & C)** CD4+ populations in PBMCs identified and visualised by FlowSOM. **C)** . The heatmap shows median marker intensities within each immune population (red represents high and blue represents low intensity). **D & F)** Representative histograms of CD4+ and CD8+ T cells depict the expression of the indicated surface molecules on T cells freshly isolated ex vivo (0h), stimulated with aCD3,aCD28,IL2 & ex vivo cultured (2 weeks), or unstimulated CTRL only cultured with IL2 (2 weeks). **E)** Representative plots showing CD8+ and CD4+ T cells respectively with or without stimulation with aCD3,aCD28, IL2 ex vivo cultured(2 weeks)*