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Building a Machine Learning Model of Murine Vaccine Response

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Research Question: Will Machine Learning methods improve the prediction of cell populations between sample groups of mice undergoing different vaccine schedules?

Objective

Develop a machine learning pipeline to identify immune cell markers that are most predictive of vaccine schedule using flow cytometry data.

Flow Cytometry and Machine Learning

- Flow Cytometry measures light scatter across ~20 fluorescence channels for ~100,000 cells for phenotyping. Machine Learning (ML) provides a scalable approach to analyze this data.
- Supervised ML is used to identify features (cell population percentage) and map them to target classes (treatment group)
- The model is iteratively trained, tested, and evaluated based on its predictive accuracy relative to the actual target classes

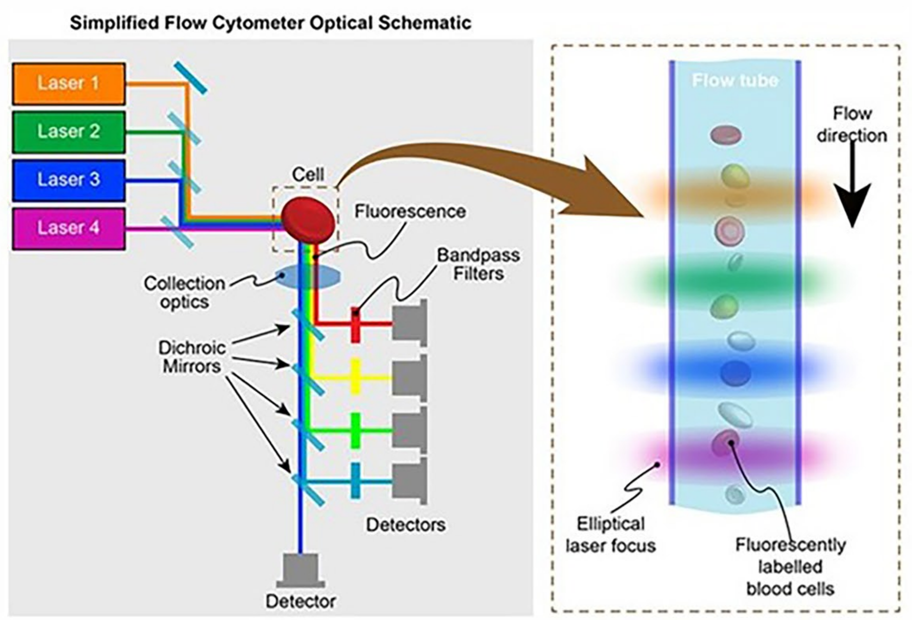

Fig 1: Flow Cytometry Graphic

Days from Dosage	
-9	Collect submandibular blood as a control group
-2	Implant pumps/tubes
1	Start pumps and give a bolus injection to other groups
7 & 14	Collect submandibular blood samples
24	Euthanize mice and harvest spleens, lymph nodes, and cardiac blood

Fig 2: Experimental Design

The Immune System and Vaccines

- The immune system is a network of cells and organs that identify and fight infections
- Vaccines inject antigens that train the immune system to fight of the disease. They can also induce inflammatory reactions, where the immune system attacks healthy cells in the body
- Important cells in the immune response can be identified through staining. For example, regulatory T-cells are positive for the CD4+ protein and FoxP3+ transcription factor
- Flow Cytometry is used to harvest such cells from organs (Spleen, Lymph Nodes) or blood to determine if an inflammatory reaction is occurring

Methods

- This project uses data from Esrafili et. al. (2025), where mice are split into four treatment groups based on frequency of vaccine dosage (Fig.2)
- FlowJo, a flow cytometry data analysis software, was used to determine the percentage cells that belonged to a certain phenotype (Fig. 3 & 4)
- Using Python libraries, Supervised ML models were trained to predict the treatment group based on 17 different phenotypes from each sample
- Sequential Feature Selection (SFS), a commonly used ML technique to enhance model performance, was applied to identify the features that were most predictive immune cell populations by minimizing classification error for the model. These results are in Fig. 5.

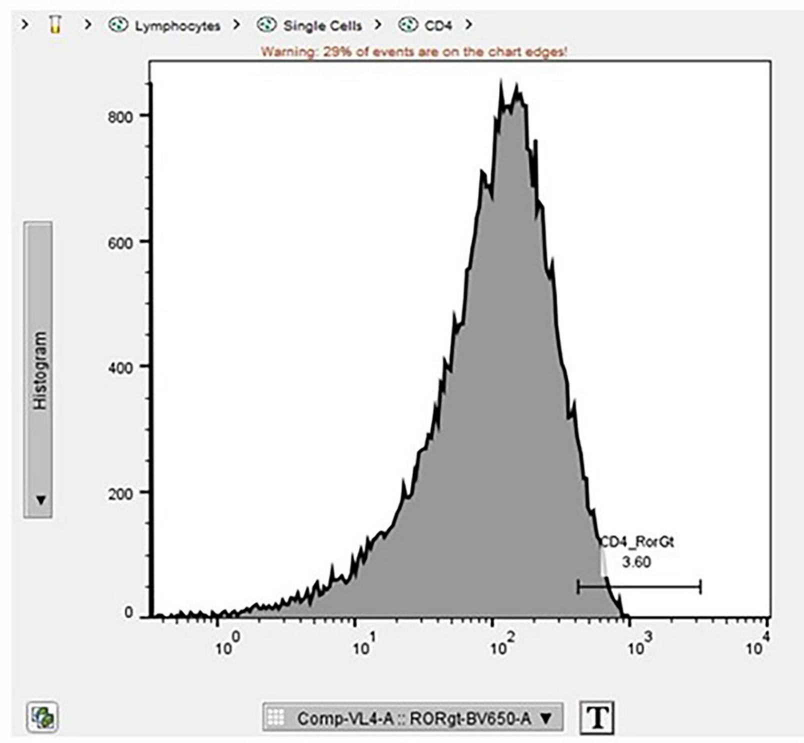

Fig 3: Gating of CD4+RorGt+ Cells

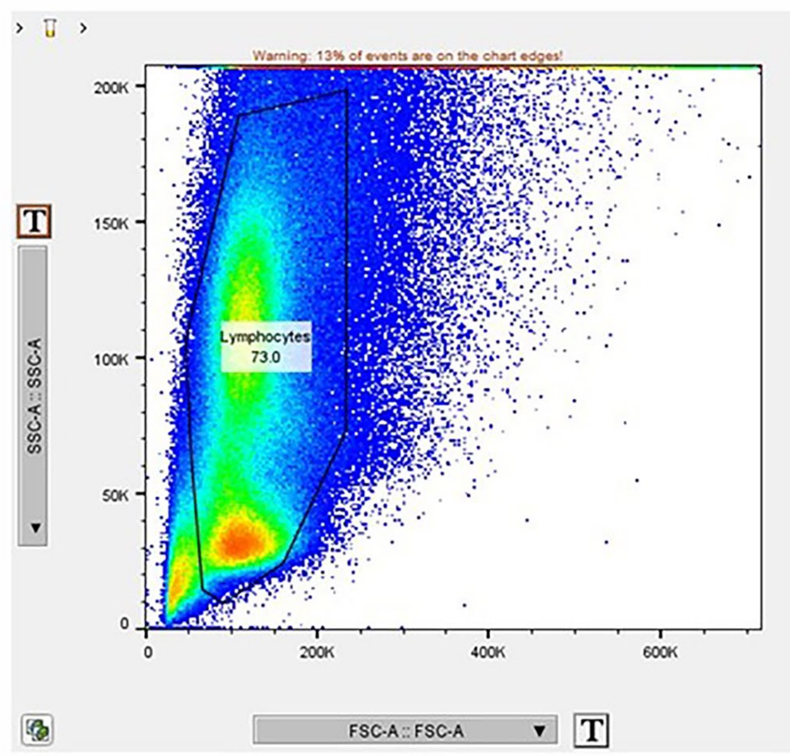

Fig 4: Gating of Non-Debris Cells

Results

- Since all samples selected CD4+FoxP3+CD25+, this means that the FoxP3+ transcription factor plays a strong role in regulating the immune response
- This aligns with the findings from Esrafili et. al. (2025), where the FoxP3+CD25+ was discovered to have the most significant impact on anti-inflammatory responses, when dosage frequency is varied
- The prominence of Gata3+ and Tbet+ populations suggest that balance between Type I and Type II populations is strongly regulated by immune response. RorGt+ presence indicates anti-microbial response
- Ki67+ cells were only selected in the speen, meaning that dosage variation triggers cell-cell interactions localized in the spleen
- The NT (No Treatment) groups selected the same exact features, which shows that there is consistency in the machine learning algorithm

	Blood	NT1	NT2	BSA Antigen	OVA Antigen	Spleen
CD4+FoxP3+CD25	■	■	■	■	■	■
CD4+Gata3+	■	■	■	■	■	
CD4+RorGt+	■	■	■	■	■	■
CD4+Tbet+	■	■	■	■	■	
CD4+FoxP3+CD25+ CD44		■	■	■	■	■
CD4+Gata3+CD44+						
CD4+RorGt+CD44+	■					
CD4+Tbet+CD44+						
CD4+FoxP3+CD25+ CD44+Ki67+						■
CD4+Gata3+CD44+ Ki67+						
CD4+RorGt+CD44+ Ki67+						
CD4+Tbet+CD44+ Ki67+						
CD4+RorGt+CD44+ CD62+						
CD4+Tbet+CD44+ CD62+						
CD4+Gata3+Ki67+						■
CD4+RorGt+Ki67+						
CD4+Tbet+Ki67+						

Fig. 5: Selected Features by Sample

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