Development of a Flow Cytometry Panel for Microglia and Immune Cell Populations in a Brain Tumor Model

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Overview

- Accurate identification of microglia versus infiltrating immune cells is critical for understanding brain tumor immunology and therapeutic responses.
- Development and optimization of a multicolor flow cytometry panel for comprehensive immune profiling in brain tumor models.
- Utilized key markers to distinguish resident microglia from peripheral immune populations.
- Optimized tissue dissociation and myelin removal to preserve epitope integrity and improve data quality.
- Validated the panel using tumor-bearing and control tissues, demonstrating clear population separation and reproducibility.
- Enables robust, scalable immune profiling for studying neuroimmune interactions and evaluating immunotherapy-driven changes within the tumor microenvironment.

Experimental Workflow

- **Tumor implantation:** GL261 cells were inoculated intracranially into C57BL/6 mice to establish an orthotopic glioblastoma tumor model
- **Tissue collection:** Whole brain, tumor plus surrounding periphery, or tumor only were harvested between Days 14–16 post-inoculation for immune profiling analysis
- **Dissociation optimization**: Samples were dissociated using either the Miltenyi Adult Brain Dissociation Kit or Miltenyi Brain Tumor Dissociation Kit.
- **Myelin removal assessment:** Each preparation was evaluated with and without myelin removal to determine its impact on sample quality and downstream flow cytometry performance.
- **Workflow goal:** These comparisons were used to optimize tissue processing conditions for accurate characterization of microglia and infiltrating immune cell populations

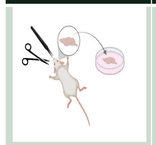
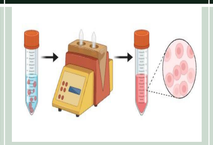
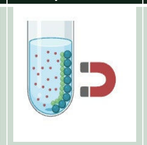
1. Harvest Brain Tissue	2. Tissue Dissociation using gentleMACs and enzymatic digestion	3. Myelin Removal by magnetic separation	4. Cell staining	5. Acquisition using BD FACSymphony
				

Figure 1: Optimized workflow with Tumor plus surrounding periphery utilizing Miltenyi Brain dissociation Kit and demyelinated using Miltenyi Myelin Removal Beads

Flow Panel Markers

Microglia	Infiltrating Monocytes	Mature Tissue Associated	Immune Cells	Antigen Presenting Cells	Other
CD45+ (low/intermediate)	CD45+ (high)	CD45+ (high)	CD45+ (high)	CD45+ (high)	Viability
CD11b+	CD11b+	CD11b+	CD3+ (T Cells)	CD11b+	CD86 (activation)
CD49d-	CD49d+	CD49d+	B220+ (B Cells)	MHCII+	CD68 (phagocytic)
CX3CR1+	Ly6C+	Ly6C-	Ly6G+ (Neutrophils)	CD11c+	
Iba1	F4/80+	F4/80+			
P2RY12		CD206 (M2)			
TMEM119					

Table 1: Flow Cytometry Panel designed to distinguish resident microglia from immune cells populations

Workflow Optimization

- Optimization of tissue processing to identify optimal dissociation kit, tissue collection and myelin removal.
  - **Tissue Collection:** Tumor plus periphery
  - **Dissociation:** Miltenyi Brain Tumor Dissociation kit
  - **Myelin removal assessment:** Demyelination

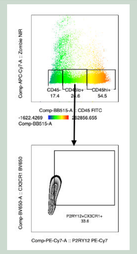
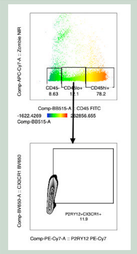
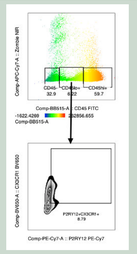
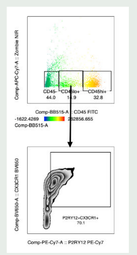
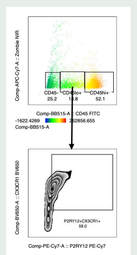
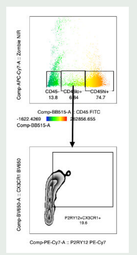
	Whole Brain	Tumor Plus Periphery	Tumor Only
Adult Brain Dissociation kit			
Brain Tumor Dissociation kit			

Figure 2: Optimization of tissue collection and dissociation kits

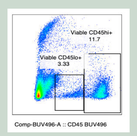
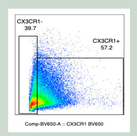
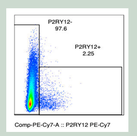
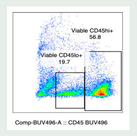
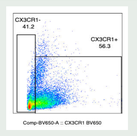
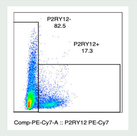
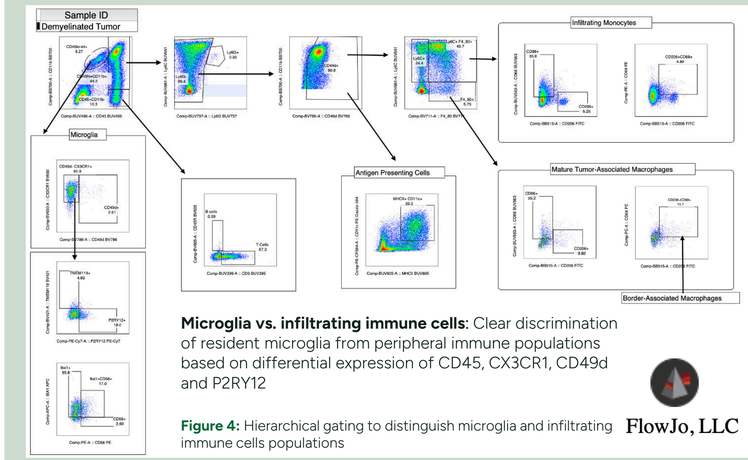
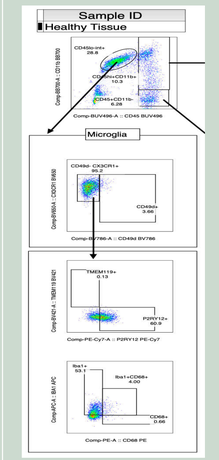
	CD45	CX3CR1	P2RY12
Myelin Present			
Myelin Removed			

Figure 3: Comparison of tissue sample with and without myelin removed

Identification of Microglia and Infiltrating Immune Cell Populations

Tumor Plus Periphery Sample	Healthy Sample
	

Microglia vs. infiltrating immune cells: Clear discrimination of resident microglia from peripheral immune populations based on differential expression of CD45, CX3CR1, CD49d and P2RY12

Figure 4: Hierarchical gating to distinguish microglia and infiltrating immune cells populations

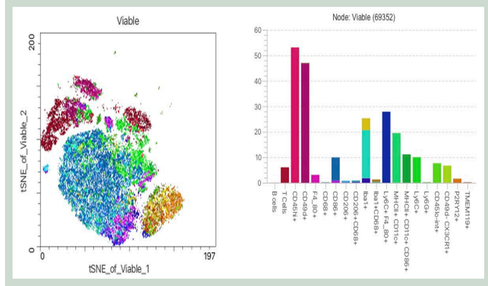
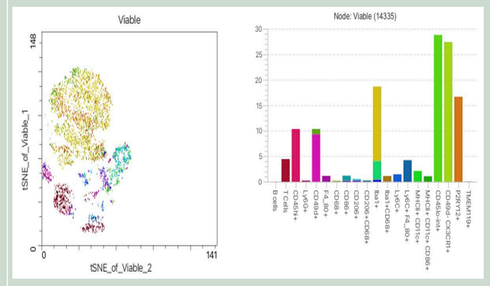
Tumor Plus Periphery Sample	Healthy Sample
	

Figure 5: t-SNE cluster plot and bar charts depicting normalized frequency (%) of events in each cluster

- **Microglia homeostatic signature:** Robust expression of the homeostatic marker P2RY12 in healthy brain tissue, with marked downregulation observed in tumor-bearing samples
- **Infiltrating myeloid populations:** Distinct identification of infiltrating monocytes and differentiation into mature tumor-associated macrophages (TAMs)
- **Border-associated macrophages (BAMs):** Identification of BAM populations characterized by a CD206⁺ phenotype
- **Phagocytic activity:** Increased CD68 expression within BAMs and macrophage populations, indicative of enhanced phagocytic function in the tumor microenvironment
- **Overall outcome:** High-resolution characterization of myeloid heterogeneity and immune infiltration within the brain tumor microenvironment

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