RESEARCH SNAPSHOT

Shocking New Insights in Immune Response from Microglia Cells

Overview

Innate immune cells, primarily brain resident macrophages and microglia, are key regulators of brain development and play a critical role in the etiology and disease progression of many brain-based conditions, including multiple sclerosis (MS), Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), traumatic brain and spinal cord injury (Lenz et al., 2018).

Microglial cells constantly survey the central nervous system (CNS) environment and as immune cells, in pathological contexts, they provide the first host defense and orchestrate the immune response. Microglia regulate brain development primarily via two routes: the release of diffusible factors aphagocytosis. Microglia phagocytose many products in the brain, including synaptic elements, living cells, dying or dead cells, and dystrophic axons. Microglia also support myelination/ oligodendrogenesis, neurogenesis, axon fasciculation, induce cell death or cell survival, and stimulate synaptic formation and maturation via the release of diffusible factors (Lenz et al., 2018). Because microglia have such a fundamental role in brain function, the potential therapeutic landscape in targeting microglia is extraordinarily broad, including developmental diseases such as autism, acquired diseases such as MS and genetic and degenerative diseases such as AD, PD and ALS.

Under pathological conditions, microglia undergo a dynamic transformation from homeostatic to disease-associated-microglial profiles. In healthy CNS, resting microglia display low or no antigen-presenting cell (APC) phenotype. In pathologic CNS, however, microglia are competently capable of presenting antigens to and activating patrolling naïve T cells, adhesion molecules, and co-stimulatory molecules expressed on activated microglia (Futabo et al., 2020).

Therapeutic targets against disease-associated-microglial induction mechanisms might serve as a way to restore the homeostatic signature of microglia and ameliorate neurodegeneration in pathologic CNS (Futabo et al., 2020). However, the goal of microglial-targeted therapy is to maintain homeostatic function and to restrain or inhibit inflammatory or disease-promoting microglia, such as those displaying an activated transcriptional profile (Butovsky et al., 2020).

KEY HIGHLIGHTS

Levitation: Targeting Neuro-immune Access

- Isolation/enrichment of microglia cells (88.3%) vs. other cell types (11.7%)
- Enrichment resulting in 94% homeostatic microglia vs activated microglia
- Maintain sensitive microglia populations with no transcriptome signature alterations.

To effectively study the role of microglia in these diseases, researchers have long tried to isolate, enrich and culture these cells, thereby modeling the disease *in vitro*. The cells are generally isolated through mechanical or enzymatic dissociation, or both, generally with Percoll gradient method and FACS sorting for enrichment. FACS is time and labor intensive, requires skilled handlers, exerts harsh pressure on cells, requires use of antibodies and does not maintain sterility of the isolated cells.

Isolate and Enrich Microglia With the LeviPrep-LeviCell System

The **LeviPrep™-LeviCell™** system, a label-free solution for sample processing, addresses all of these challenges through fast, simple, and extremely gentle microglia / macrophage enrichment without altering transcriptional signatures.

Microglia enrichment with the LeviCell system is illustrated in Figure 1. In Figure 1A, the entire LeviPrep workflow (PN 1005001) is represented starting with the tissue through the cell harvest from the LeviCell system.

The protocol begins with a 30 minute cold enzymatic and mechanical digestion of the minced brain tissue. Then, the enzyme is inactivated and the sample is washed. At this point, the user obtains a single cell suspension of their cells that contains myelin and red blood cells (RBCs), when using a non-perfused brain. To enrich for microglial cells, a Percoll gradient which simultaneously removes the myelin and the RBCs from the sample (Figure 1B) is required *(Adult Brain Protocol, Doc #90-00073).*

Efficient Microglia Enrichment Post LeviPrep-LeviCell Workflow

We isolated cells using our LeviPrep workflow and the Percoll gradient method (Figure 1) using label-free viable LeviCell enrichment. Single cell RNA sequencing was performed on the collected cells using 10x Genomics Chromium™. Downstream data analysis (Figure 2) shows that the LeviPrep-LeviCell workflow is able to enrich microglia (88.3%) as compared to other cell types (11.7%) containing neurons, oligodendrocytes and astrocytes.

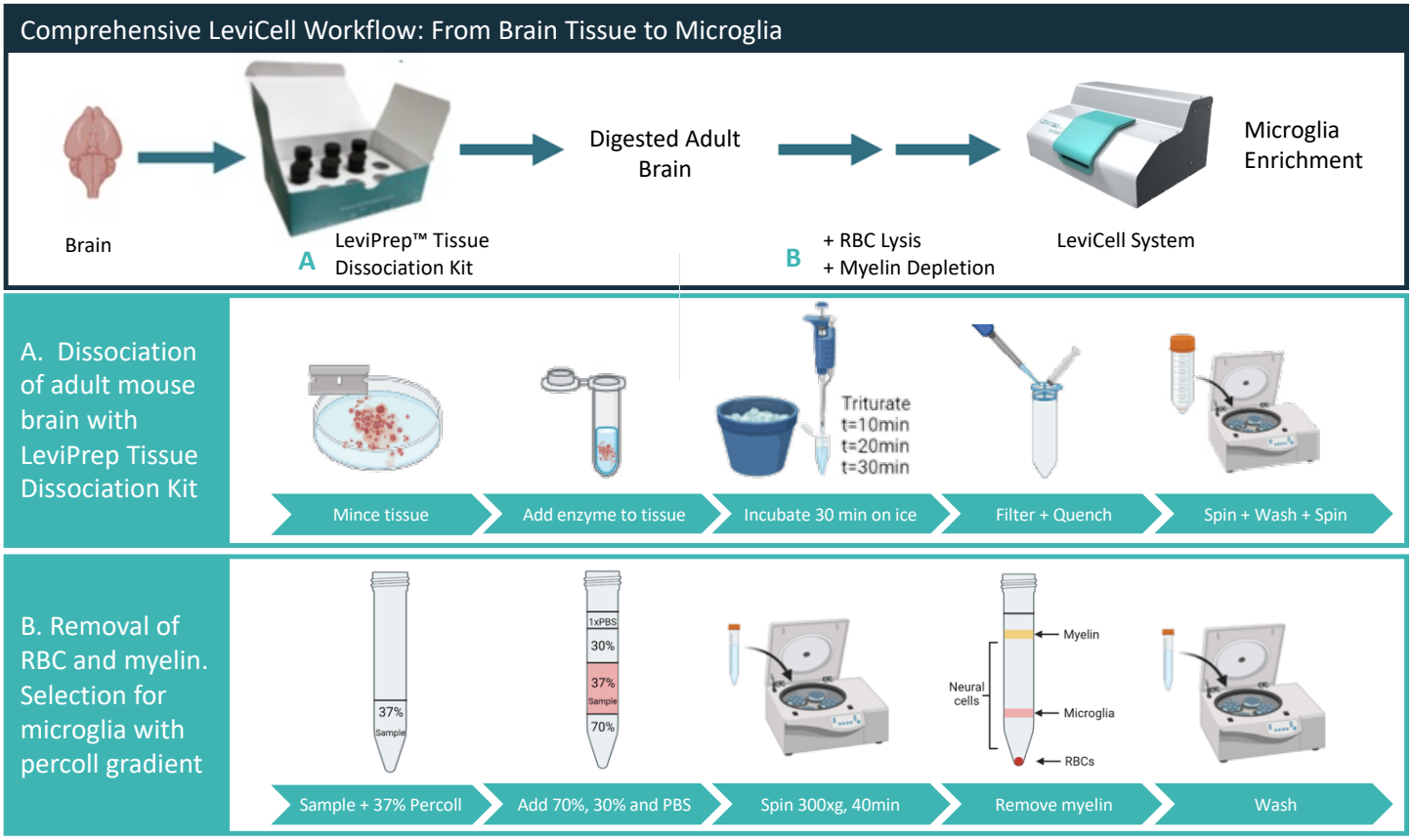

Figure 1. Schematic representation of the workflow when using a non-perfused adult mouse brain. *(A) General workflow from the tissue, LeviPrep protocol and preparation of samples for enrichment with the LeviCell system. (B) Steps to follow after the LeviPrep protocol and before LeviCell, when microglia enrichment is desired (Nikodemova et al., 2012). Created with BioRender.com. RBC: Red Blood Cell*

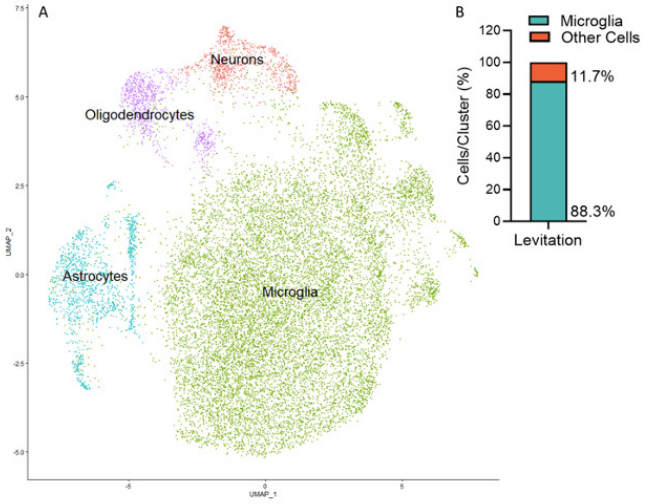

Figure 2. Efficient microglia enrichment post LeviPrep-LeviCell workflow. *(A) Representative UMAP demonstrating different cell types profiled by single cell sequencing after levitation. (B) Quantification of percentage of microglial cells per cluster obtained after LeviPrep-LeviCell workflow (88.3%) as compared to other cell types (11.7%).*

LeviCell System Does Not Alter Transcriptomic Profile

Upon further subsetting of the microglial cluster from the dataset, we were able to distinguish microglia subtypes based on their cellular state, namely: homeostatic, activated (proliferative and inflammatory microglia) and infiltrating macrophages. As seen in Figure 3, the LeviPrep-LeviCell workflow leads to an enrichment of homeostatic microglia as compared to proliferating or inflammatory microglial states.

Leveraging Levitation For Sensitive Cell Enrichment

Many researchers in the field of neuro-degenerative diseases have started to focus their research on the neuro-immune axis.

The LeviCell workflow provides a quick, easy and efficient way to enrich for viable cells. This **Levitation Technology** can also be used to enrich sensitive cell type populations like microglia. These cells after levitation are ideally suited for elucidating the disease interaction involving the neuro-immune axis as researchers can determine the effect of certain proteins or drugs on

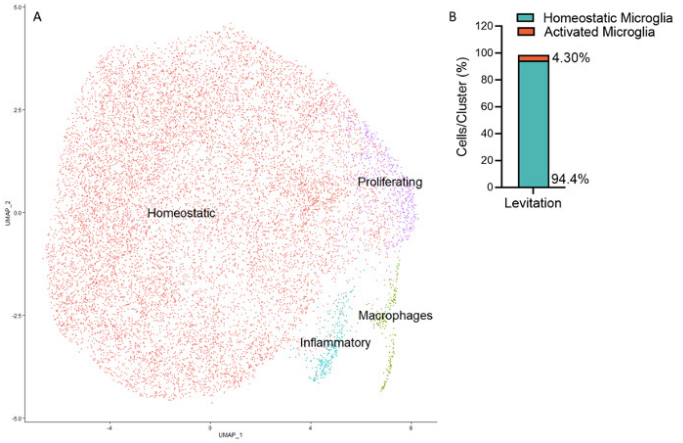

Figure 3. LeviPrep-LeviCell workflow enriches homeostatic microglia. *(A) Representative UMAP demonstrating different microglia types profiled using single cell sequencing after levitation. (B) Quantification of percentage of homeostatic microglia and activated microglia (proliferating and inflammatory) per cluster*

activation of microglia. On the other hand, standard sorting methods can lead to an increased expression of activated microglial markers and therefore, possibly would not respond to any treatments similar to that of homeostatic microglia. Hence, the Levitation Technology helps maintain the microglial transcriptome signature which is of great importance for drug development and targeted therapeutics.

Viable cell enrichment using Levitation Technology on the LeviCell system is a powerful application and can be leveraged in various fields. Here, we have demonstrated its utility working with highly sensitive cell types such as microglia, but additional examples include CRISPR positive clone selection, cancer modeling such as patient derived xenografts engraftment, separating cells with aggregates and cells with intrinsic differences in densities and many more.

References

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