Analysis of immune cells from inflamed mouse brain and spinal cord

Background

Neurodegeneration implies the slow and progressive dysfunction and loss of neuronal structures and functions in the central nervous system (CNS), representing the primary pathological features of acute and chronic conditions such as Alzheimer's disease and Parkinson's disease, neurotropic viral infections, stroke, paraneoplastic disorders, traumatic brain injury, and multiple sclerosis[1]. Despite the different etiologies, a common feature of neurodegenerative pathologies is chronic activation of the immune system.[1,2]

Inflammatory responses in the CNS are initiated by microglia, the resident macrophages of the CNS, and astrocytes.[3,4] Infiltration of immune cells from the periphery such as macrophages, T cells, and B cells can further contribute to inflammation in the CNS.[5,6] Yet, the detailed mechanisms of neuroinflammation and their detrimental as well as beneficial effects are still elusive. To better understand the inflammatory processes, it is essential to analyze the molecular and functional characteristics of immune cells involved. This often requires dissociation of the inflamed tissue into single cells. However, some of the most important immune cell markers such as CD4, CD8, and CD19 are highly sensitive towards proteases and the epitopes are usually degraded during dissociation.

This application note describes the procedure to dissociate inflamed neural tissue using the Multi Tissue Dissociation Kit 1 and the gentleMACS™ Octo Dissociator with Heaters for subsequent flow cytometric analysis and isolation of immune cells. This process combines automated mechanical tissue dissociation with mild enzymatic treatment based on pre-defined enzymes to ensure preservation of immune cell–specific epitopes. After tissue dissociation, cell debris and erythrocytes are depleted using Debris Removal Solution and Red Blood Cell Lysis Solution, enabling optimal detection of target cells by flow cytometry. The use of REAfinity™ Recombinant Antibodies allows reproducible and background-free flow cytometry analysis. Different immune cell populations can be further isolated using MACS® Technology.

Materials and methods

Animals

CD-1 IGS mice with induced experimental autoimmune encephalomyelitis (EAE) were used throughout this study. Brain and spinal cord were resected according to standard procedures.

Tissue dissociation

Perfused and non-perfused whole brain and spinal cord from EAE mice were dissected and dissociated into single-cell suspensions using the Multi Tissue Dissociation Kit 1 in combination with the program "37C_Multi_F" on the gentleMACS Octo Dissociator with Heaters. This combination of mechanical and enzymatic dissociation provides gentle conditions to preserve cell surface epitopes and cell functionality.

Debris and erythrocyte removal

After dissociation, cell debris was eliminated using the Debris Removal Solution. Subsequently, erythrocytes were removed using Red Blood Cell Lysis Solution. If perfusion was performed, depletion of erythrocytes was carried out optionally.

The entire procedure of tissue dissociation and debris /erythrocyte removal is described in detail in the protocol "Dissociation of inflamed neural tissue using the Multi Tissue Dissociation Kit 1", available at www.miltenyibiotec.com

To compare the preservation of protease-sensitive epitopes, some samples of perfused whole brain were additionally dissociated using papain instead of the enzymes contained in the Multi Tissue Dissociation Kit 1. Conditions of the papain-based dissociation were otherwise exactly the same as for the kit.

The resulting single cells were resuspended in PB buffer, which consists of phosphate-buffered saline (PBS, pH 7.2) containing 0.5% bovine serum albumin, and the cell number was determined. PB buffer was prepared by diluting MACS BSA Stock Solution at a ratio of 1:20 with PBS.

Flow cytometry analysis

Expression of the following molecules was analyzed by flow cytometry after dissociation of spinal cord and perfused whole brain: CD3, CD4, CD8a, CD11b, CD11c, CD19, CD20, CD25, CD27, CD45, CD45R (B220), CD49b, CD68, F4/80, Ly-6G, Ly-6C, and NK1.1.

Cells were kept cold and pre-cooled solutions were used to prevent capping of antibodies on the cell surface and non-specific cell labeling. Cell suspensions were labeled with antibodies according to the corresponding data sheets and analyzed on a MACSQuant® Analyzer 10.

Results

Automated dissociation of mouse brain tissue and spinal cord derived from EAE mice

We developed a protocol for the efficient dissociation of inflamed rodent neural tissue using the Multi Tissue Dissociation Kit 1 and the gentleMACS™ Octo Dissociator with Heaters. Whole brain and spinal cord from EAE mice were used for development of the protocol. To optimize the percentage of viable cells, debris and erythrocytes were depleted after dissociation. This process led to a substantial increase in the percentage of intact Ter-119$^-$ cells (fig. 1A and B). Dissociation of one EAE mouse brain yielded between 5.6×10^6 and 1.3×10^7 total cells with 78–80% viable cells. In comparison, one spinal cord yielded 6.4×10^6 to 7.9×10^6 total cells and a viability rate of 75–81% (fig. 2).

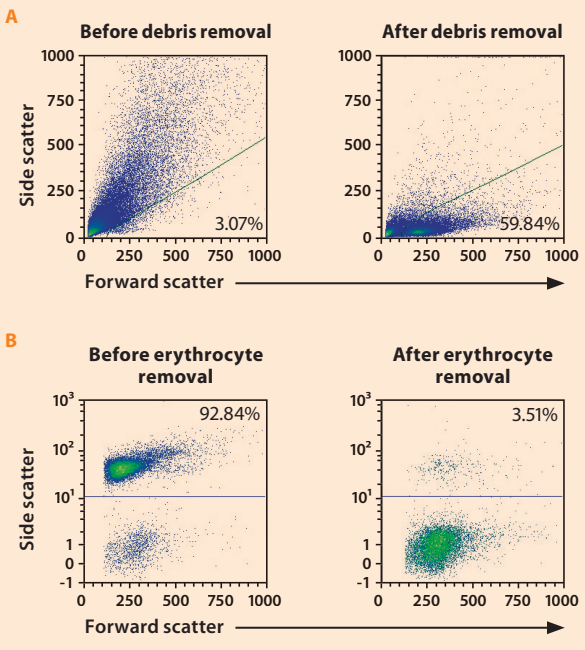

Figure 1: Effective removal of debris and erythrocytes increases the percentage of intact Ter-119$^-$ cells after dissociation of inflamed brain tissue from EAE mice. Plots show representative flow cytometry data depicting the enrichment of intact Ter-119$^-$ cells after debris (A) and erythrocyte (B) removal from dissociated EAE mouse brain.

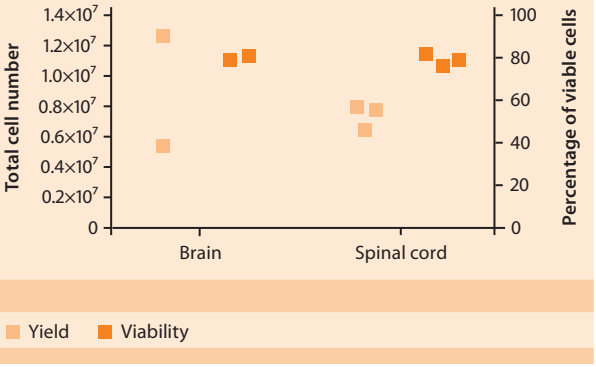

Figure 2: Yield and viability of cells obtained after dissociation of inflamed neural tissue from EAE mice. Results are shown for dissociation of one brain (n = 2) and one spinal cord (n = 3). Yield values refer to the left y-axis and viability values to the right y-axis.

Preservation of epitopes for successful identification of immune cell populations from EAE mouse brain tissue and spinal cord

The dissociation procedure was developed particularly to enable the effective generation of single-cell suspensions from inflamed neural tissue, i.e., brain and spinal cord, and yet preserve protease-sensitive markers. Perfused whole brain from EAE mice was dissociated using the Multi Tissue Dissociation Kit 1 compared to a papain-based dissociation protocol. Expression of protease-sensitive immune cell epitopes was analyzed by flow cytometry (table 1). The papain-based dissociation procedure resulted in complete degradation of highly sensitive epitopes such as CD8a, CD19, and CD45R, and partial degradation of CD4 (fig. 3A), whereas the Multi Tissue Dissociation Kit 1 did not lead to degradation of these molecules (fig. 3B).

Surface epitopes	Antibody clone	Papain-based dissociation	Multi Tissue Dissociation Kit 1
CD3	REA641	●	●
CD4	REA604	●	●
CD8a	REA601	●	●
CD11b	REA592	●	●
CD11c	REA754	●	●
CD19	REA749	●	●
CD20	REA294	n.a.	●
CD25	REA568	●	●
CD27	REA499	●	●
CD45	REA737	●	●
CD45R (B220)	REA755	●	●
CD49b	REA541	●	●
CD68	FA-11	●	●
F4/80	REA126	●	●
Ly-6G	REA526	●	●
Ly-6C	IG7.G10	●	●
NK1.1	PK136	●	●

● Stable ● Moderate sensitivity ● Strong sensitivity

Table 1: Integrity of immune cell marker epitopes analyzed after dissociation of neural tissue. Preservation of immune cell marker epitopes was compared between a papain-based dissociation protocol and the Multi Tissue Dissociation Kit 1. Epitope sensitivity towards the respective dissociation protocol is specified as stable, moderate, or strong.

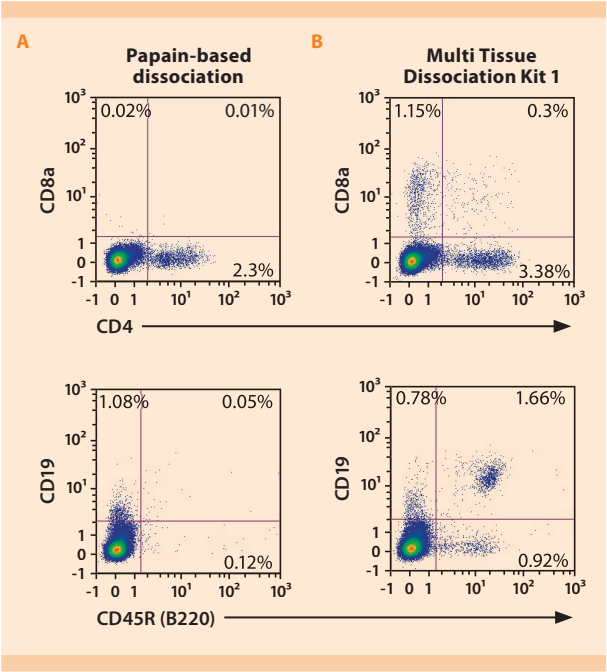

Figure 3: Preservation of protease-sensitive immune cell marker epitopes from EAE mouse brain by the use of the Multi Tissue Dissociation Kit 1. Flow cytometry analysis of protease-sensitive immune cell marker epitopes was performed after dissociation by a papain-based protocol (A) or the Multi Tissue Dissociation Kit 1 (B). Debris, dead cells, and doublets were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Analysis of immune cell subpopulations from EAE mouse brain and spinal cord

The Multi Tissue Dissociation Kit 1 protocol allowed for subsequent flow cytometry analysis of major immune cell subpopulations from perfused whole brain and spinal cord from EAE mice. Microglia, macrophages, and monocytes were distinguished based on CD45 and CD11b, neutrophils based on CD11b and Ly-6G, T cells based on CD3, CD4, and CD8a, B cells based on CD19 and CD45R, and NK cells based on CD3 and NK1.1 (fig. 4A and B). Spinal cord tissue contained larger percentages of immune cells in comparison to whole brain (fig. 5). Dissociation of one EAE mouse brain yielded between 6% and 12% CD45$^+$ (n = 2), cells while dissociation of one spinal cord yielded 19–21% CD45$^+$ cells (n = 3). Yields and percentages of different immune cell subpopulations may differ depending on the status of the disease.

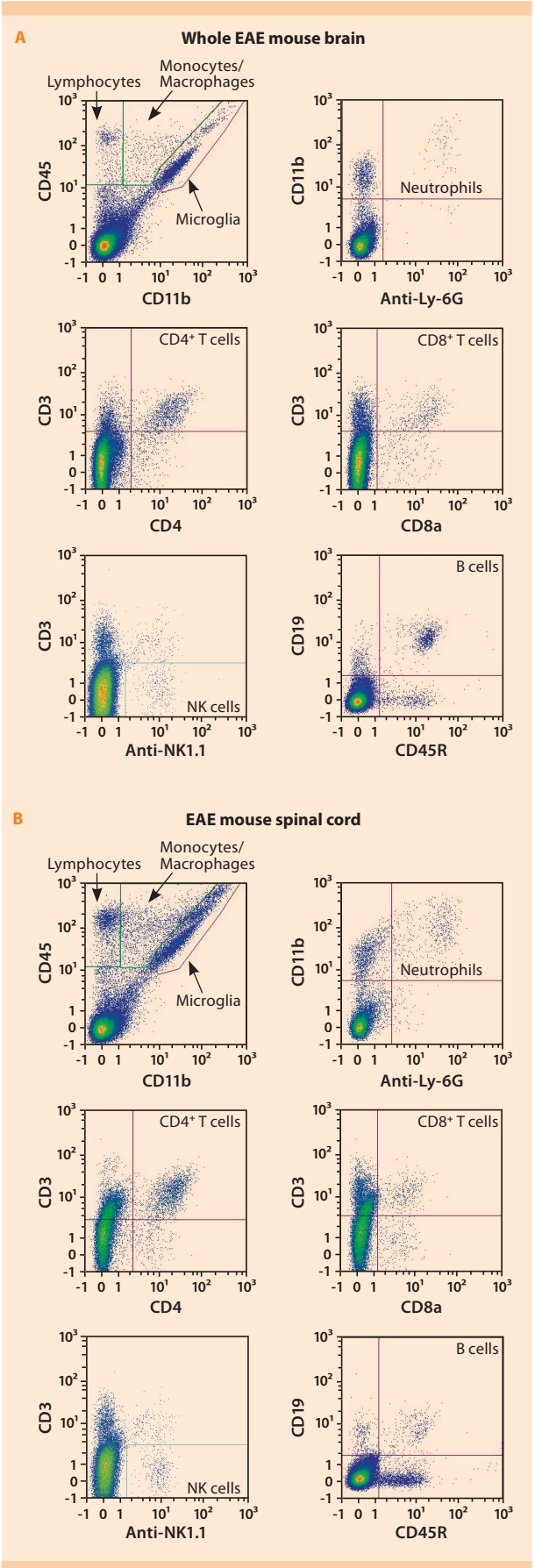

Figure 4: Flow cytometry analysis of immune cell subpopulations from brain and spinal cord from EAE mice. Representative flow cytometry data for the identification of immune cell subpopulations after dissociation of (A) brain (n = 2) and (B) spinal cord (n = 3) from EAE mice by the use of the Multi Tissue Dissociation Kit 1. Debris, dead cells, and doublets were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

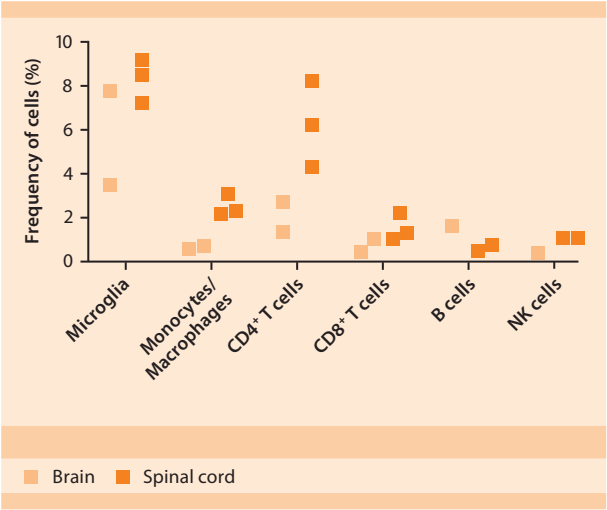

Figure 5: Frequency of immune cell subpopulations among CD45$^+$ cells from dissociated brain and spinal cord from EAE mice. Frequencies of immune cell subpopulations were determined by flow cytometry after dissociating brain (n = 2) and spinal cord (n = 3) from EAE mice with the Multi Tissue Dissociation Kit 1.

Conclusion

- Inflamed brain and spinal cord can be effectively dissociated into single-cell suspensions using the Multi Tissue Dissociation Kit 1 and the gentleMACS™ Octo Dissociator with Heaters, followed by removal of cell debris and erythrocytes for optimized recovery of immune cell subtypes.
- This protocol does not only allow for optimal recovery of immune cells, but also ensures epitope integrity of protease-sensitive immune cell markers for downstream applications, such as immunophenotyping and immunomagnetic isolation or flow sorting.
- These tools greatly expand the options for the analysis and isolation of immune cells from inflamed brain and spinal cord and thus facilitate sophisticated preclinical studies on neurodegenerative diseases.

References

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