A novel automated dissociation procedure for inflamed neural brain and spinal cord allows detection and isolation of immune cells

Introduction

Acute and chronic neurodegenerative diseases such as multiple sclerosis, Parkinson’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis, stroke, or brain injury result in a slow and progressive dysfunction of the central nervous system (CNS) with the loss of neuronal structure and function as the primary pathological feature. Despite various causative triggers, a common feature is the presence of proteases and the epitopes are usually degraded during the process.

As epitope stability is the prerequisite for reliable detection of target cells from the dissociated tissue, we developed a new automated dissociation procedure by using enzymatic treatment with mechanical dissociation using thegentiMACS™ Octo Dissociator with Heaters. Tissue dissociation is followed by a novel process for removal of debris and erythrocytes, which is crucial for subsequent successful immune cell detection. This optimized process allowed for the efficient dissociation of brain and spinal cord derived from EAE mice with low amounts of cell debris and led to reproducible numbers of viable immune cells. Protease-sensitive markers such as CD4, CD8a, CD19, and CD45R (B220) were preserved and immune cell lineages, including T cells, B cells, macrophages, monocytes, and NK cells, could be identified. Furthermore, immune cell subsets were successfully isolated from the dissociated tissue based on MACS™ Technology. In summary, we present a novel standardized technology to generate single-cell suspensions from inflamed rodent neural tissue that preserves epitope integrity and facilitates analysis and isolation of infiltrated immune cell subsets.

Results

Preservation of epitopes for successful identification of immune cell subpopulations from EAE mouse brain

The novel dissociation procedure was developed to enable an efficient dissociation of inflamed brain and spinal cord and to preserve protease-sensitive markers such as CD4, CD8a, CD19, and CD45R (B220). Perfused whole brain from EAE mouse was dissociated using the Multi Tissue Dissociation Kit (MTDK) in combination with the new protocol. For comparison, a standard dissociation procedure was applied. Debris and erythrocytes were subsequently removed using the Debris Removal Solution and Red Blood Cell lysing Buffer, respectively (Miltenyi Biotec). Cells were stained with CD4, CD8a, CD19, and CD45R (B220)-specific, fluorochrome-conjugated antibodies for flow cytometry analysis. Debris, dead cells, and doublets were excluded from the analysis based on scatter signals and propidium iodide fluorescence. The optimized dissociation process did not lead to degradation of the protease-sensitive markers (fig. 2B). In contrast, a papain-based dissociation procedure resulted in complete degradation of CD4a and CD8a and partial degradation of CD19 and CD45R (fig. 2A). To summarize, the novel dissociation procedure for inflamed neural tissue allowed for the detection of intact immune cell lineages, i.e., CD4+ and CD8a+ T cells, B cells, macrophages, monocytes, and NK cells. For detection of most of the antigens, RAFlinity™ Resistant Antibodies were used (table 1).

Conclusion

- We present a novel dissociation procedure that allows the automated dissociation of inflamed brain or spinal cord and preserves epitope integrity of protease-sensitive immune cell markers such as CD4, CD8, CD19, and CD45R (B220). 
- The optimized procedure allows detection and isolation of immune cells from dissociated inflamed rodent neural tissue and enables detailed analysis of different immune cell lineages, e.g., CD4+ and CD8a+ T cells, B cells, monocytes, macrophages, monocytes, and NK cells.
- Purified immune cells can be applied to study their molecular and functional characteristics to better understand the inflammatory processes in neurodegenerative diseases.