Comparing the Influence on Surface Markers of Primary Isolated Splenocytes: Enzymatic Treatment vs. Mechanical Dissociation with TissueGrinder

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Introduction

Efficient isolation of primary splenocytes is pivotal for immunological investigations. The preservation of cell surface marker is particularly important, as they mediate key immunological functions and form a prerequisite for many downstream applications, including flow cytometry and cell separation.[1,2] Besides mechanical tissue dissociation, proteolytic enzymes are being used more frequently to obtain single cells, that may induce changes in the expression, consequently affecting immune cell functions and misleading results.[3,4] In the present study, we compared the effects of enzymatic and mechanical digestion on the surface markers CD25, CD62L and CD27 of primary murine splenocytes.

Methods

Spleen tissue was collected and dissociated through a metal mesh (UMM Interne Anzeige: I-22/32). Splenocytes (1x10^6 cells/ml) were cultured in RPMI-1640 (ThermoFisher Scientific) and incubated with the enzymes collagenase I, IV, V, D from Clostridium histolyticum (Sigma-Aldrich) and dispase from Bacillus polymyxa (Stemcell) for the indicated time periods with different enzyme concentrations[4] (5% CO_2, 37°C). For further reference, automated dissociation methods, including the enzymatic GentleMACS® system (GM) from Miltenyi Biotec and mechanical dissociation using TissueGrinder[5] (TG) were performed. Cells were collected by centrifugation and stained with anti-mouse FITC CD27, PE CD62L and APC CD25 (BioLegend) for assessment of surface expression via Flow Cytometry (Cytek Northern Lights). In addition, cell viability, cell yield and cell stress, by comparing GSH/GSSG-Ratios (Promega), were carried out.

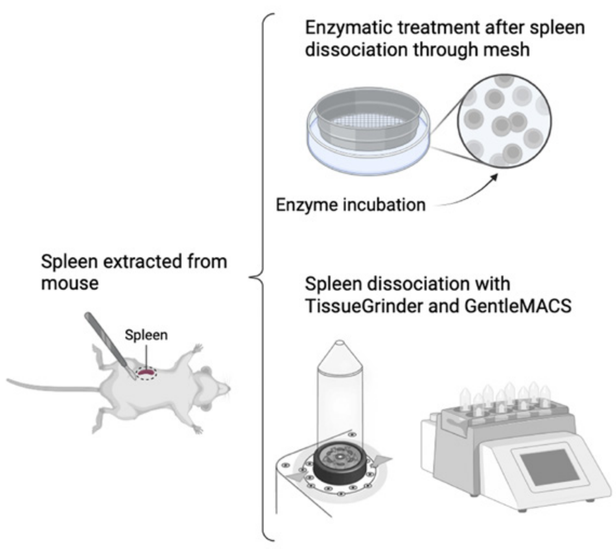

Fig.1 Experimental Design
Visual depiction of the process steps involved in spleen dissociation employing both enzymatic and mechanical methods. This illustration was generated using BioRender.com.

Results

Significant differences were observed between enzymatic digestion and mechanical dissociation concerning surface marker expression.

Enzymatic treatment after dissociation through mesh

Due to enzymatic treatment, a significantly reduced surface marker expression of CD27 and CD62L could be recorded with increasing incubation time of collagenase IV, V and dispase, respectively. This distinction was particularly prominent during the enzymatic incubation period of 30 minutes. The partial elevation in surface marker expression, e.g. the activation marker CD25, illustrates the stimulation-induced division of T cells (Fig. 2A). Similar outcomes can be observed with an increase in enzyme concentration, while the enzymes have minimal impact on CD25, as shown in Fig. 2B.

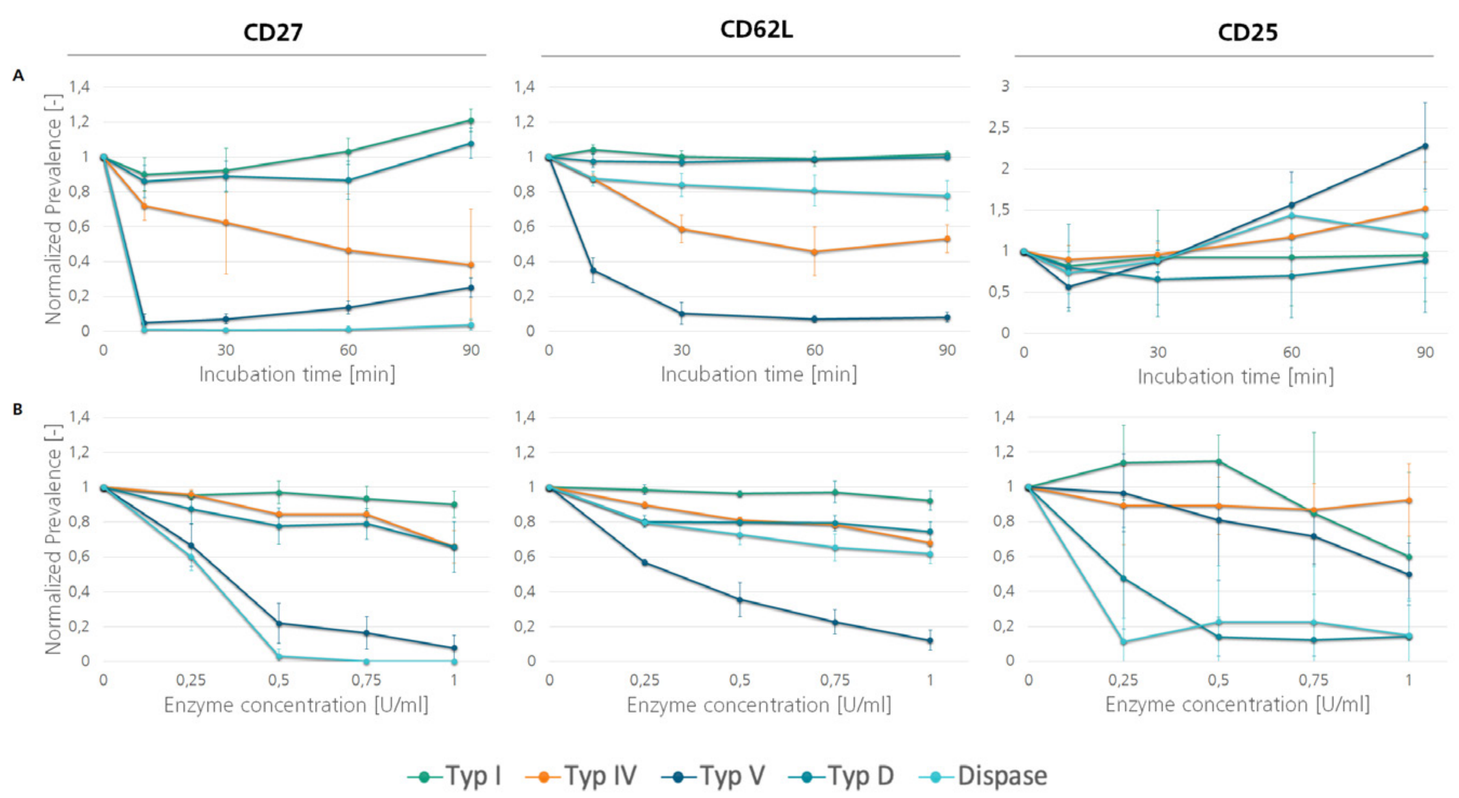

Fig.2 Influence of enzymatic incubation duration and diverse enzyme concentrations on the expression levels of CD25, CD27, and CD26L in splenocytes following treatment with collagenase (Typ: I, IV, V, D) and dispase. A: Splenocytes were incubated with collagenase I, IV, V, D and dispase (0.5 U/ml) for the indicated time periods. The prevalence is presented as normalized means ±SD (n=3) in relation to the initial incubation time. B: Splenocytes were incubated with various concentrations (0.25-1.0 U/ml) of collagenase I, IV, V, D and dispase for 10 min. The prevalence at the indicated enzyme concentrations is presented as normalized means ±SD (n=4) in relation to the initial enzyme concentration 0 U/ml.

Spleen Dissociation: TissueGrinder vs. GentleMACS

Comparing the automated dissociation methods (Fig. 3), it is evident that the non-enzymatic approach with TG yields higher prevalences of CD27 and CD62L in comparison to the enzymatic method employing GM. In addition, there is an observable trend indicating a higher cell yield with comparable viability when utilizing the TG method. Considering cell stress, both systems show a higher GSH/GSSG-Ratio than the control (dissociation through mesh), which consequently confirms a more gentle dissociation. The turnaround time from sample to single cells is ~15 minutes for the TG and ~40 minutes for the GM.

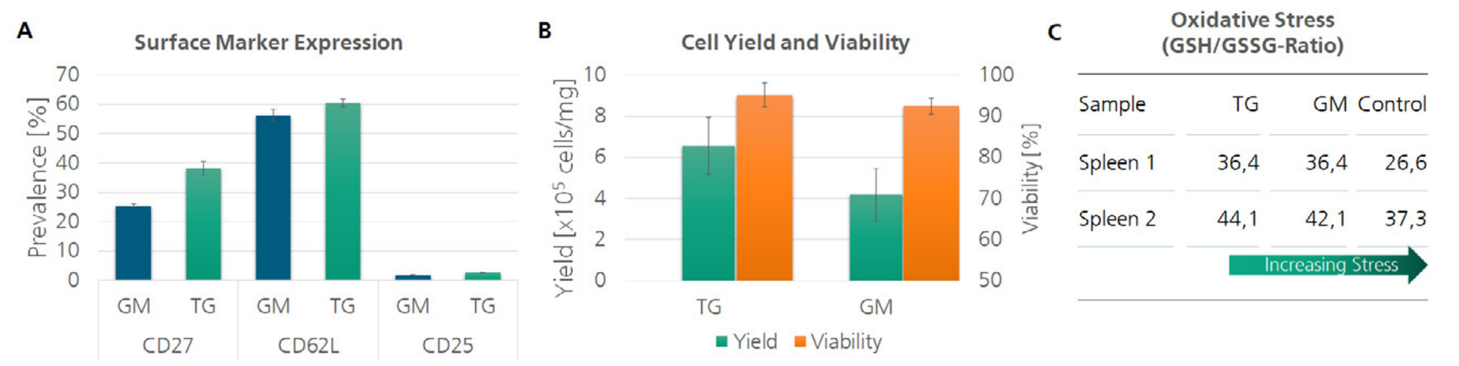

Fig.3 Comparison between automated mechanical dissociation using the enzyme-free TissueGrinder and the enzymatic GentleMACS system regarding surface marker expression, cell yield, viability and cell stress. A: Splenocytes were analyzed for their surface markers immediately after dissociation. For a direct comparison, the spleen sample was bisected into two equal halves, with each half undergoing either dissociation with TG or GM. The experiment was conducted in triplicate. B: Cell yield, expressed as cells per milligram (cells/mg), and viability were assessed through trypan blue exclusion using a hemocytometer. Data are given in triplicate as mean values with their standard deviation. C: Oxidative Stress is given in GSH/GSSG-Ratio. To facilitate a direct comparison, one-third of the spleen sample 1 and 2 was subjected to the respective method of dissociation.

Conclusion

The choice of isolation technique significantly influences the preservation of surface markers on primary splenocytes. Enzymatic tissue dissociation can have profound effects on the expression of certain surface markers such as CD27 and CD62L with serious consequences for functional effects and target cell isolation. In contrast, enzyme-free mechanical dissociation using the TissueGrinder offers the advantage of:

- Fast and gentle dissociation process
- Preserving certain surface markers like CD62L and CD27
- Cell yields, viability and cell stress are comparable to enzyme-based methods

Researchers should tailor their tissue dissociation method based on their experimental needs to ensure accurate downstream analyses and interpretations.

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