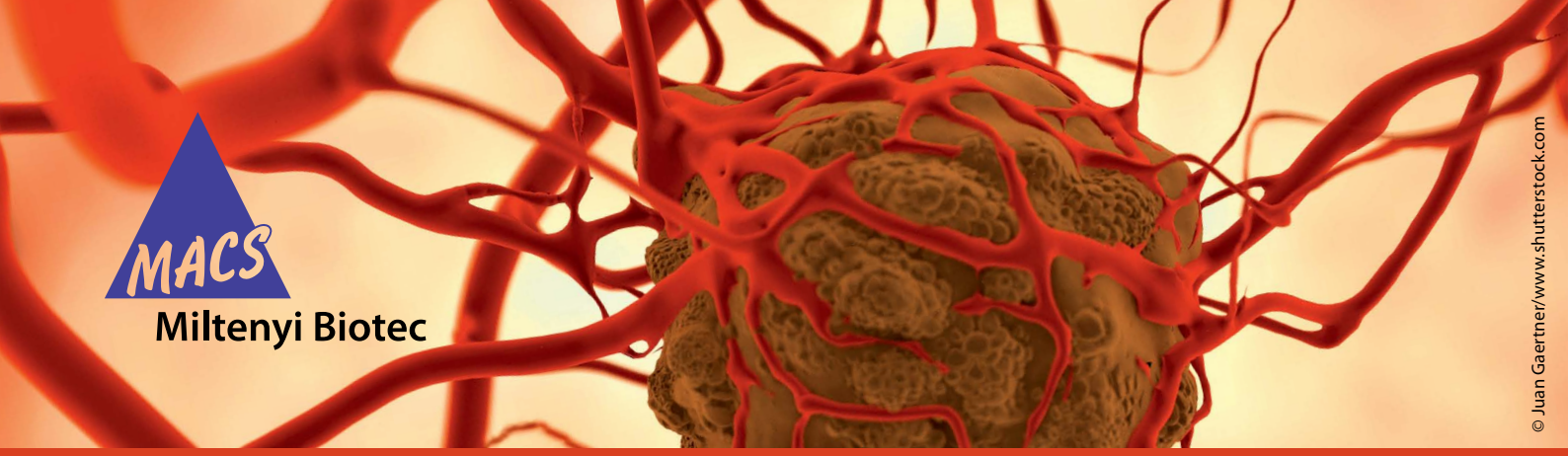

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Dissociation of mouse glioblastoma

Isolation and analysis of CNS-infiltrating lymphocytes

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Background

Murine and human glioblastoma (GB) cell populations contain a fraction of cells with stem cell-like features, and it has been proposed that only this population may be responsible for glioma recurrence. Moreover, GB cell populations enriched in glioma stem-like cells (GSCs) can give rise to gliomas that closely resemble the original tumor but that are rather different from the experimental gliomas generated by brain injections of serum-driven established cell lines.
The glutamate-aspartate transporter GLAST is a radial glia marker that is highly expressed in GL261 GSCs obtained from established cultures of murine gliomas.
The expression of GLAST on GB cells supports the idea that GLAST could be a good target for GB immunotherapy.
We show that immunization with GLAST-derived peptides effectively promotes specific antitumor responses.
The data suggest that the absence of autoimmune reactions and toxicity in this experimental model is associated with a chemotactic gradient that facilitates the homing of immune cells to the tumor site.

This note describes the procedure to isolate and to analyze CNS-infiltrating lymphocytes using the gentleMACS™ Dissociator and the MACSQuant® Analyzer.

Materials and methods

Materials

- Tumor Dissociation Kit, mouse
- PEB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution 1:20 with autoMACS® Rinsing Solution. Keep buffer cold (2–8 °C).
- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes
- MACSmix™ Tube Rotator in combination with an incubator at 37 °C
- Centrifuge
- MACSQuant Analyzer
- CD4-PE-Cy™5 antibodies, mouse
- CD3-FITC antibodies, mouse
- CD8-FITC antibodies, mouse

Methods

1. Prepare enzyme mix of the Tumor Dissociation Kit, mouse according to the data sheet.
2. Remove glioma-bearing hemispheres from the mouse and cut it into small pieces of 2–4 mm.
3. Transfer the tissue into the gentleMACS C Tube containing the enzyme mix.
4. Tightly close C Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
5. Run the gentleMACS Program **m_impTumor_02**.
6. After termination of the program, detach C Tube from the gentleMACS Dissociator.
7. Incubate sample for 40 minutes at 37 °C with continuous rotation using the MACSmix Tube Rotator.
8. Attach C Tube upside down onto the sleeve of the gentleMACS Dissociator and run the gentleMACS Program **m_impTumor_03**.
9. After termination of the program, detach C Tube from the gentleMACS Dissociator and perform a short spin up to 300×g to collect the sample at the bottom of the tube.
10. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 70 µm, placed on a 15 mL tube.
11. Wash cell strainer with 10 mL of RPMI 1640 and centrifuge cell suspension at 300×g for 7 minutes. Aspirate supernatant completely.

12. Suspend single-cell suspensions in PEB buffer for labeling and flow cytometry.
13. Stain cells in PBS for 30 minutes at 4 °C with the respective antibodies.
14. Perform flow cytometry acquisition on the MACSQuant Analyzer and analyze with the MACSQuantify™ Software.

Results

The results of the present study support the contention that GLAST may constitute a glioma antigen which immune responses can be efficiently induced without major safety concerns.

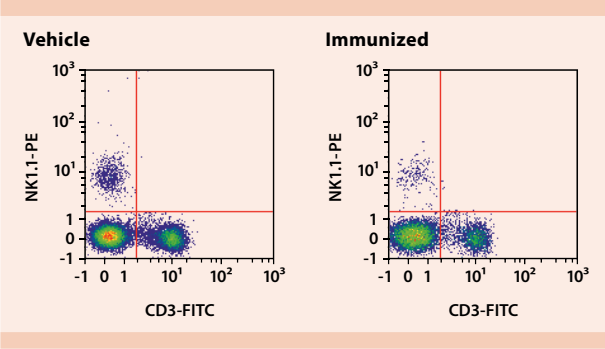

Figure 1: Flow cytometry performed on splenocytes from immunized mice showed that the percentages of $CD3^+$ T cells were significantly increased when compared with controls.

Conclusion

Isolation and analysis of CNS-infiltrating lymphocytes can be accomplished with ease using the gentleMACS Dissociator and the MACSQuant Analyzer.

References

1. Pellegatta, S. *et al.* (2012) Immunotherapy against the radial glia marker GLAST effectively triggers specific antitumor effectors without autoimmunity. OncoImmunology 1: 1–10.
2. Pellegatta, S. *et al.* (2006) Neurospheres enriched in cancer stem-like cells are highly effective in eliciting a dendritic cell-mediated immune response against malignant gliomas. Cancer Research 1: 10247–10252.

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