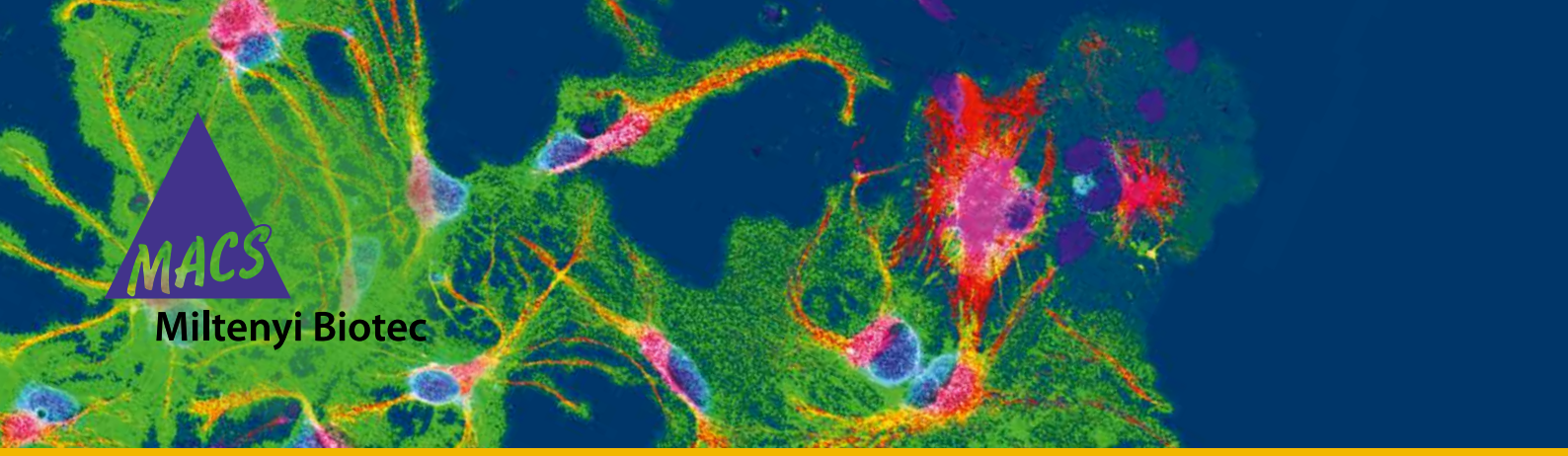

Dissociation of adult mouse brains to isolate CD3+ T cells

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Background

Glioblastoma multiforme (GBM) represents the most frequent and deadliest primary brain tumor. Aggressive treatment still fails to eliminate deep brain infiltrative and highly resistant tumor cells. Human Vγ9Vδ2 T cells, the major peripheral blood γδ T cell subset, react against a wide array of tumor cells and represent attractive immune effector T cells for the design of antitumor therapies[1].
It was aimed to provide a pre-clinical rationale for immunotherapies in GBM based on stereotaxic administration of allogeneic human Vγ9Vδ2 T cells and notably to evaluate whether Vγ9Vδ2 T cells, amplified from peripheral blood leukocytes (PBLs) of healthy donors, survived and moved within the mice-brain parenchyma[2].
This protocol describes the procedure to dissociate adult mouse brain tissue using the gentleMACS™ Octo Dissociator with Heaters to isolate CD3+ T cells.

Materials and methods

Materials

- gentleMACS Octo Dissociator with Heaters
- gentleMACS C Tubes
- Adult Brain Dissociation Kit, mouse and rat
- Centrifuge with swing buckets
- MACS® SmartStrainers (70 µm)
- D-PBS buffer
- Bovine serum albumine (BSA), 0.5%
- NSG mice (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ), 8–10 weeks old (Charles River Laboratories; Wilmington, MA, USA)
- CD3 antibodies conjugated to APC, human

Methods

NSG mice were stereotaxically injected with 1×10^7 human CD3+ T cells. Total brain cells were isolated on day 1, 3, or 7 after injection. Each brain was manipulated separately.

1. Remove the mouse brain.
2. Wash the brain in D-PBS buffer and cut it into 8 sagittal slices using a scalpel.
3. Dissociate brain samples using the Adult Brian Dissociation Kit in combination with the gentleMACS Octo Dissociator with Heaters according to the protocol.
4. After the debris and red blood cell removal steps, cells were washed twice in D-PBS buffer.
5. Resuspend cells in D-PBS buffer with 0.5% BSA at 1×10^6 cells/mL.
6. Label cells with CD3 antibodies conjugated to APC and analyze by flow cytometry.

Results

The transferred human CD3+ T cells injection can be found in adult mouse brains after day 1, 3, and 7 of injection.
The frequency of human CD3+ T cells in mouse brains decreased from 50% at day 1 to 10% at day 7.
The isolation of CD3+ T cells from adult mouse brain can be accomplished with ease using the the gentleMACS Octo Dissociator with Heaters in combination with the Adult Brain Dissociation Kit.
Moreover, CD3+ T cells can also be activated and expanded. Following sorting and antigenic stimulation indicate that T lymphocytes can survive within the brain (data not shown).

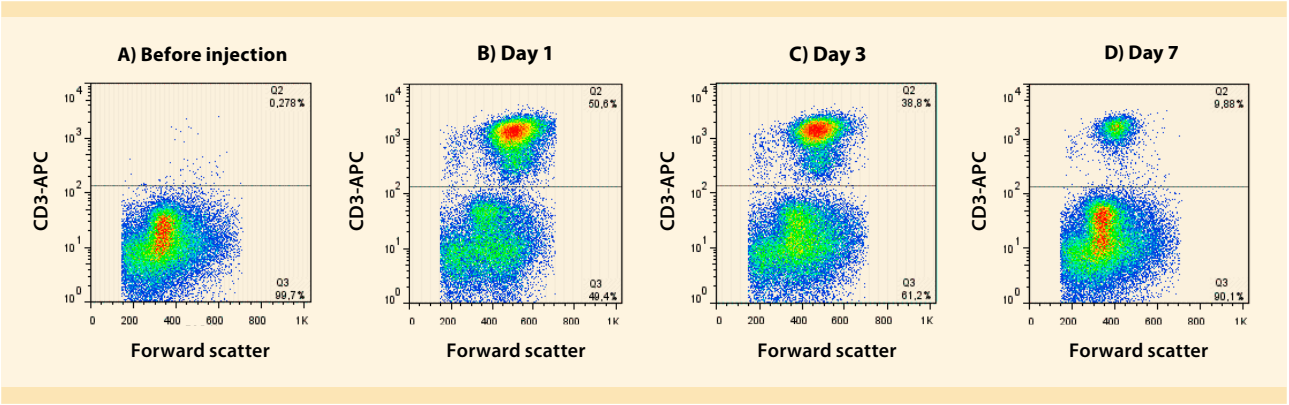

Figure 1: Flow cytometric analysis before (A) and after injection of human CD3⁺ T cells in adult mouse brains at day 1 (B), day 3 (C), and day 7 (D).

References

1. Silva-Santos, B. *et al.* (2015) γδ T cells in cancer. Nat. Rev. Immunol. 15 (11): 683–691.
2. Jarry, U. *et al.* (2016) Stereotaxic administrations of allogeneic human Vγ9Vδ2 T cells efficiently control the development of human glioblastoma brain tumors. Oncoimmunology: In press.

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