

Imaging immune response in vivo: Cytolytic action of genetically altered T-cells directed to glioblastoma multiforme

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INTRODUCTION: Despite emerging therapies patients with glioblastoma multiforme survive only 1-2 years after initial diagnosis. Recent conceptual and technological advances in the field of tumor immunology are starting to offer therapies based on redirected immune response. Among them, genetically modified T-cells that express a chimeric T-cell receptor with a membrane-bound IL-13 cytokine (zetakine) have been proposed [1]. This membrane bound zetakine can selectively bind the IL-13 $\alpha 2$ receptor (IL13Ra2) that is expressed exclusively by high grade glioblastoma in the central nervous system. This enables activation of zetakine expressing T-cells to be independent of major histocompatibility complex (MHC)-antigen presentation and therefore not restricted to an individual's MHC type, but rather to a specific antigen (IL13Ra2). In vitro studies have shown that activation of the chimeric zetakine receptor upon contact with IL13Ra2⁺ glioma cells is sufficient to trigger T-cell effector functions ultimately leading to cytolytic destruction of tumor cells. In this work we investigated the use of MRI to determine parameters that can aid in the in vivo study of interactions between zetakine expressing T-cells and the glioblastoma, and ultimately to be translated for clinical evaluation of proposed treatment.

METHODS: Male NOD-*scid* mice were used for all experiments. Mice were anesthetized with ketamine/xylazine (132/8.8 mg/kg) prior to stereotaxic injections. Glioblastoma cells (U87 cell line) were suspended in PBS (1 X 10⁵ cells/ μ l) and injected bilaterally: 2 mm lateral and 0.5 mm anterior to Bregma; 1 μ l was injected at 2.5 mm depth from dura, and 1 μ l at 2.25 mm depth from dura. T-cells were transformed to express IL-13 zetakine as previously described [1]. Mice were injected, with 2 X 10⁶ IL-13-zetakine T-cells (N=4) eleven days after glioblastoma cells to the same coordinates, right side only. Two control groups received PBS (N=3), and non-specific T-cells (N=4). MRI was performed on a 7.0 T Bruker Biospin system using a 2 cm birdcage coil, one day prior (-1 day), one, two and three day post (+1 day, +2 days and +3 days) T-cell/PBS injection. Each animal was imaged with a T₂-weighted multi-echo spin echo sequence (five 0.5 mm thick slices, TR/TE=3000/10.6-148.4 ms, 14 echoes, 117X117 μ m² resolution, 4 averages) and diffusion-weighted imaging (five 0.5 mm-thick slices, TR/TE=3000/29 ms, big delta=12 ms, small delta=5 ms, with three b-value=0, 500 and 1000, 127X163 μ m² resolution, 2 averages). T1-WI spin echo (nine 0.5 mm thick slices, TR/TE=500/10.6 ms, 117X117 μ m² resolution and 8 averages) images were acquired following contrast agent (Magnevist). Transverse relaxation time constants (T₂) and apparent diffusion coefficients (ADC) were calculated on a pixel-by-pixel basis from the corresponding exponential fits using CCHIPS software [2]. Post-contrast hyperintense volumes in T1-WI images were determined using an automated segmentation routine, CCHIPS [2]. At day 14 all mice were perfused with 4% paraformaldehyde and brain slices were processed for histology (hematoxylin and eosin (H&E) staining and CD45 for T-cells). ANOVA was used for statistical analysis.

RESULTS: Significantly increased T₂-values (p<0.05) were present in the ipsilateral vs. contralateral hemisphere (56±2 vs. 47±2 ms) of mice treated with zetakine expressing T-cells 1-2 days following the injection (Fig. 1A, 2). No significant differences were present in mice treated with PBS or non-specific T-cells (Fig. 1A). While T₂-values returned back to near normal at day 3 post injection (Fig. 1A, 2), ADC values significantly increased (p<0.05) in the ipsilateral vs. contralateral (1.05±0.07 vs 0.72±0.03 X 10⁻³ mm²/s) hemisphere, at 2-3 days post injection of mice treated with zetakine expressing T-cells. There was no significant increase in ADC values in mice treated with PBS or non-specific T-cells (Fig. 1B). Only mice treated with specific T-cells had significant reduction of hyperintense signal volume on T1-WI (p<0.05) in the ipsilateral vs. contralateral hemisphere (0.6±0.3 vs. 2.4±0.4 ml). Histological examination of the tumor bed confirmed that the area of elevated T₂- and ADC-values on MRI matched the area of tumor destruction (Fig. 2).

CONCLUSIONS: Since glioblastoma multiforme is rapidly progressing tumor, it is highly desirable to effectively predict treatment outcome. Elevated T₂-values at day 1-2, and increased ADC-values at 2-3 days following zetakine T-cell injection have correlated well with successful cytolytic destruction of glioblastoma by zetakine expressing T-cells evident on histology. Both of these parameters can be easily monitored by MRI in clinical settings to aid better prognosis. Furthermore, due to the increased blood brain barrier (BBB) permeability associated with progression of glioblastoma, full or partial restoration of the BBB integrity may be additional MRI indicator of tumor regression. In conclusion our data supports the use of IL-13 zetakine T-cells in clinical trials for patients with glioblastoma multiforme.

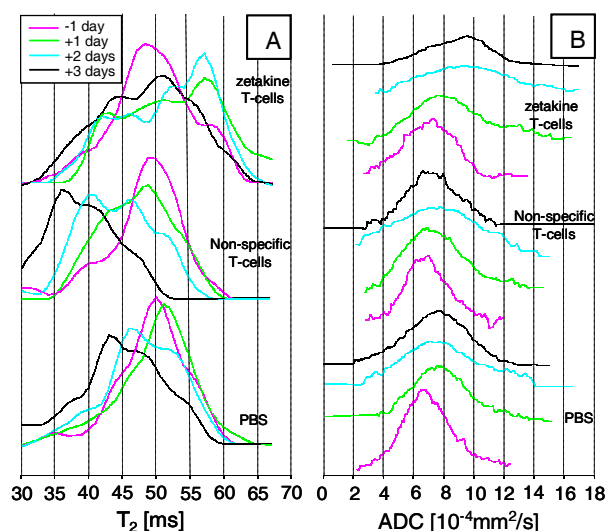
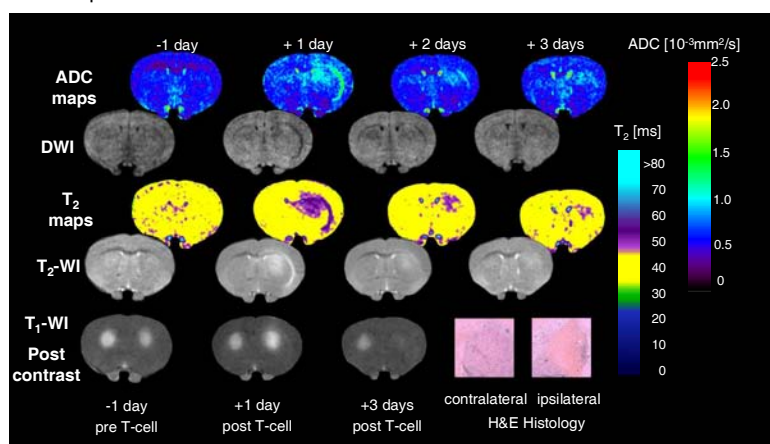


Figure 1. A stacked histograms corresponding to (A) T₂- and (B) ADC-values for ipsilateral hemisphere, different days and treatments. Histograms were calculated from the region of interest based on the increased signal on T1-WI, and averaged among the animals in the same treatment group.

Figure 2. Representative ADC maps, DWI, T₂-maps, T₂-WI and T1-WI post contrast images of the animal treated with zetakine expressing T-cells and matching H&E histology from the ipsilateral and contralateral tumor bed. Color scale represent different T₂- and ADC-values.



REFERENCES:

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