Magnetically Labeled Sensitized Splenocytes to Identify Glioma by MRI

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Background: Dendritic cell-based vaccination therapy against recurrent glioma is in early clinical trials. A patient's dendritic cells are pulsed *ex vivo* with a glioma cell lysate collected from that patient's tumor. Following administration of the cell-lysate-pulsed dendritic cells in patients there is evidence that the administered dendritic cells initiate immunogenic activity against glioma cells, delaying recurrence and/or decreasing recurrence rate. The accumulations of primed dendritic and sensitized T-cells that occur at the site of glioma are indicative of an immunogenic reaction and may be exploited to delineate the boundary of the disease. The 9L gliosarcoma cell line has been shown to initiate an immunogenic reaction when transplanted peripherally or intracerebrally in a syngenic rat. We hypothesize that after glioma implantation in syngeneic rats both dendritic cells and T-cells present in the spleen would be sensitized and recognize glioma in the second recipient rats but splenocytes from control rats would not recognize tumors in the second recipient rats. The purpose of this study was to investigate the feasibility of imaging the migration and incorporation of magnetically-labeled sensitized splenocytes (T-cells) in an experimental glioma model (9L).

Methods: Splenocytes were collected from tumor bearing or control host rats, labeled with ferumoxides-protamine sulfate (FePro) and injected intravenously to recipient rats bearing an intracranial 9L tumor. Three days later, multi-echo T2*-Weighted (TEs of 5, 10, 15, 20, 25, and 30 msec and a TR of 3000 msec, 32 mm FOV, 1 mm slice thickness, 256x256 matrix, and NEX = 2) and 3D gradient echo (TR=100 msec, TE=9 msec, 10º flip angle, 32x32x16 mm^3 FOV, 256x192x128 matrix, and NEX = 2) MRI were obtained by a 7 Tesla MR system. T2* maps were created from the T2*-weighted images. Signal intensity and T2* values in the tumors and contralateral brain were determined by operator-defined hand drawn ROIs from 3D and T2* maps, respectively. Following *in vivo* MRI, rats were euthanized and then perfused with PBS and 3% paraformaldehyde. Collected whole brains were further fixed in 3% paraformaldehyde mixed with 30% sucrose for 24 hours and snap frozen for frozen sectioning. Sections were stained for CD45 markers to determine the accumulated hematopoietic cells. Prussian blue staining was performed to detect the iron positive cells. Before injection to tumor bearing rats, splenocytes collected from both tumor bearing and control rats were subjected to flowcytometric analysis to determine the population of cytotoxic T-cell and different markers of dendritic cells.

Results: Both 3D and T2*-weighted MRI showed low signal intensity areas in the tumors as well as at the margin of the tumors in rats that received labeled splenocytes collected from another tumor bearing rats (sensitized splenocytes). Iron positive as well as CD45 positive cells were present at the corresponding sites of low signal intensities seen on MRI. In contrast, no definite low signal intensity on MRI was observed in the tumors in rats that received labeled splenocytes collected from control rats (non-sensitized splenocytes). Similarly, no positive cells were observed using Prussian blue and CD45 staining in the control studies. Flowcytometric analysis showed significantly increased number of both helper and cytotoxic T-cells in splenocytes collected from tumor bearing rats. Population of dendritic cells were also showed tendency to increase in tumor bearing rats.

Conclusion: The studies presented demonstrate that the immunogenic reaction can be exploited to delineate glioma boundary using the localization and MR imaging of magnetically labeled sensitized splenocytes (or T-cells). The results have the potential to improve the efficacy of conventional diagnosis and therapies as well as to guide the delivery of new immunologic approaches to brain tumor treatment.

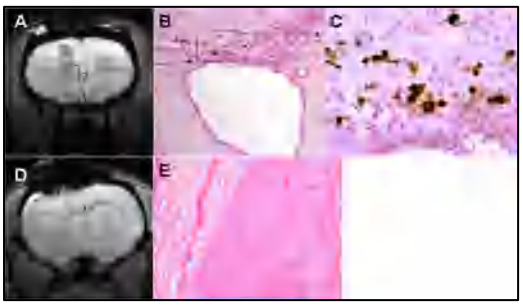

Figure 1: Gradient echo (TE=10ms) MRI and the corresponding DAB enhanced Prussian blue stained section of the tumor injected with sensitized splenocytes from a tumor bearing rat (A,B,C) and the tumor injected with splenocytes from a control rat (D, E). Please note the signal intensity changes in the tumors (A vs D). DAB enhanced Prussian blue staining shows multiple iron positive cells at the corresponding sites of the low signal intensity in the tumor (B = 10x and C = 40x). There is no iron positive cells seen in the tumor injected with splenocytes from a control rat (E = 10x).

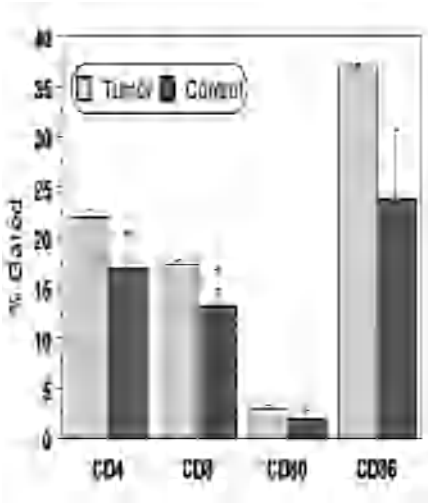

Figure 2: Flowcytometric analysis of splenocytes collected from the control and tumor bearing rats. Percent of helper (CD4) and cytotoxic (CD8) T-cells and antigen presenting cells (dendritic cells, CD80 and CD86) in the spleens collected from the tumor bearing (9L) and control rats. Number of helper and cytotoxic T-cells were significantly higher (*p<0.05) in the rats with implanted 9L tumors. Although there were no significant differences of antigen presenting cells between the tumor bearing and control rats, both CD80 and CD86 positive cells were higher in the tumor bearing rats. n=4 for tumor and n=3 for control. Data are presented as mean ± SEM.