Vaccination of Recurrent Glioblastoma Patients with Autologous Tumor Cells Activates Both T-Lymphocyte and Humoral Immune Responses

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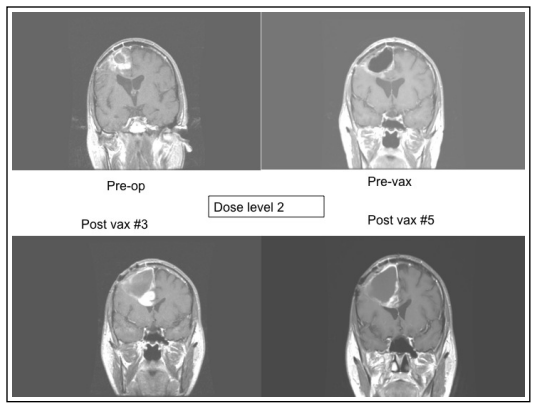
Introduction

We have previously reported the safety and feasibility of vaccinating recurrent malignant glioma patients with irradiated autologous cells mixed with irradiated GM-K562 cells. Here, we report the impact of vaccination on the expression of T-lymphocyte co-stimulatory and co-inbhibitory molecules as well as on regulatory T-lymphocytes. Furthermore, we explored the impact of vaccination on patient humoral immune responses with particular attention to antibody titers against molecules associated with tumor angiogenesis.

Methods

Blood was collected from 9 of 10 treated patients at regular intervals and submitted for immune analysis. 5-color flow cytometry was employed after staining of whole blood by an immunophenotyping antibody panel. Antibody titers to tumor-associated antigens were measured by ELISA of patient plasma – all assays were performed in duplicate. Treatment-associated change in percent expression of various markers was analyzed via the non-parametric sign-rank test. $P < 0.05$ was considered statistically significant.



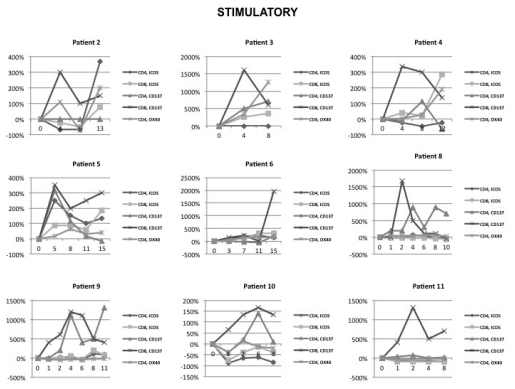
Results

In patients undergoing craniotomy for recurrent malignant glioma, vaccination with irradiated autologous tumor cells and irradiated GM-K562 cells was associated with statistically significant increases in CD4+ T-lymphocyte expression of costimulatory molecules OX40 and 4-1BB (CD137). CD8+ T-lymphocytes saw increased expression of 4-1BB as well. Likewise, there were increases in CD4+ T-lymphocyte expression of CTLA-4, PD1, and FOXP3 – each of which is associated with negative immune regulation and are targetable with existing monoclonal antibodies. 7 of 9 patients had marked treatment-driven increases in antibody titers to Angiopoietin 2, which may represent a novel glioma vasculature-associated antigen. Antibody titers to Angiopoietin 1, Hepatocyte Growth Factor (HGF), and Platelet Derived Growth Facotor (PDGF) were increased after vaccination as well. Antibodies to these cytokines were not detectable in unvaccinated patients with recurrent malignant glioma.

Conclusions

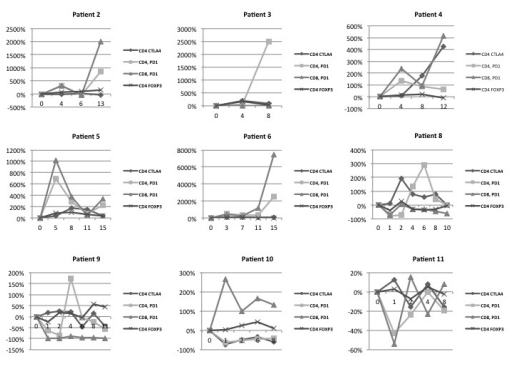
Vaccination with irradiated autologous tumor cells mixed with irradiated GM-K562 cells in patients with recurrent malignant glioma is biologically active and activates both T-lymphoctes and humoral antitumor immunity. Vaccine-induced expression of costimulatory molecules may provide the rationale for combination therapies with either co-stimulatory receptor agonist or co-inhibitory receptor antagonist molecules.

Per Cent Change in T-lymphocyte expression of costimlulatory molecules



Statistically significant increased expression of OX40, 4-1BB (CD4 and CD8) was seen after vaccination. Expression levels of these T-lymphocyte activation markers tended to peak then subside as the vaccination schedule ended.

Per Cent Change in CD4 and CD8 T-lymphocyte expression of "co-inhibitory" molecues and Foxp#



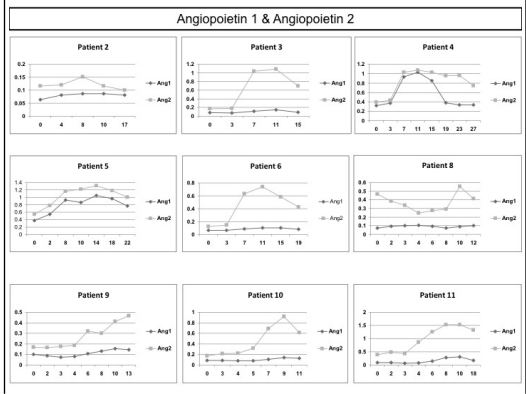
Expression of molecules associated with negative immune regulation was increased by vaccination as well.

Relative Change in Absorbance of patient plasma via ELISA for various angiogenic cytokines

	Angiopoietin 1	Angiopoietin 2	HGF	PDGF
Patient 2	-	-	+	-
Patient 3	+	+++	+	+
Patient 4	+++	+++	+	+
Patient 5	+++	+++	+	+++
Patient 6	-	+++	+	+
Patient 8	-	-	+	+
Patient 9	-	+++	-	+
Patient 10	-	+++	+	+++
Patient 11	+	+++	+	+

Autologous Glioma Cell vaccination leads to novel and increasead antibody responses to cytokines associated with tumor angiogenesis

Change in Antibody Response to Angiopoietins 1 and 2 over time



Antibody titers peak several weeks after initiation of vaccination, and then decline

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