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Modulation of Microglia Cell Phenotype by Semi-Allogeneic Cancer Vaccines

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Abstract

Glioblastoma multiforme (GBM) is the deadliest brain tumor and is one of a group of tumors referred to as gliomas. GBMs make up approximately 15% of all primary brain tumors. Even with the standard therapies of surgical resection with radiation and chemotherapy, the prognosis for patients is poor. Semiallogeneic vaccines (SAV) have demonstrated efficacy in animal models of GBM; however, the mechanisms associated with this effect are not well understood. In the current study, we examined the potential impact of SAV on the regulation of microglia in the brain. The impact of interferon-γ (IFN-γ) and vaccine mediated pathways was investigated for their effect on microglia and the modulation of phenotypic expression. *In vitro* studies revealed that IFN-γ directly and SAV treatment of dendritic cells and T-cells can mediate the production of interferon-γ that can allow for the induction of M1 microglia to facilitate tumor regression. *In vitro* studies revealed that phenotypic modulation of microglia by interferon-γ either by direct stimulation or mediated through T-cells contributes to the therapeutic effect on GBMs.

Keywords

Glioblastoma, Immunotherapy, Cancer, Vaccine, T-Cell, Microglia

1. Introduction

Glioblastoma multiforme (GBM) is the deadliest brain tumor and is a subgroup of tumors referred to as gliomas. GBMs are classified as a Grade IV (most serious) astrocytoma, constituting approximately 15% of all primary brain tumors [1]-[6]. Each year, more than 3000 Americans are diagnosed with GBMs [7]. GBMs develop from the astrocytes that support nerve cells, primarily in the cerebral hem-

ispheres, but can develop in other parts of the brain, brainstem, or spinal cord. Standard treatment for GBM is surgery, followed by combined radiation therapy and chemotherapy. GBM's capacity to invade and infiltrate surrounding brain tissue makes complete resection difficult without severe loss of healthy tissue. After surgery, combined chemoradiation is used to kill leftover tumor cells and to delay recurrence. Temozolomide was approved by the FDA in 2005 as a standard of care chemotherapeutic treatment of adult GBM; subsequently, the FDA approved Avastin (Bevacizumab) for treatment of GBM, commonly administered in combination with Temozolomide. Nevertheless, with standard of care therapy, the median survival of children and adults with GBM is 15 months, and the 5-year survival rate is approximately 10% [7] [8]. This tumor ultimately takes the life of nearly every affected patient.

The capacity of T cells to recognize allogeneic major histocompatibility complex (MHC) molecules as intact structures on the surface of foreign cells is called direct T-cell allorecognition and is responsible for the powerful immune reactions associated with transplant rejection, a phenomenon called "alloagression" [9]-[14]. This is primarily due to the ability of allogeneic stimulation to mobilize up to 10% of all T lymphocytes, compared with a precursor T-cell frequency of between 10^{-4} and 10^{-5} for most common antigens. At the same time, each of the lymphocytes activated through direct allorecognition will also recognize a specific antigenic peptide presented in the context of a self-MHC molecule (MHC restriction). Cross-reactivity between alloantigens and self MHC-restricted antigens can be harnessed to target tumor antigens [8].

Microglial activation is an essential event in the defense of the brain parenchyma from neurological diseases [15]. Additionally, microglia can switch to different functional or polarized states, depending on the type and severity of an injury [16]. Numerous studies have provided evidence that microglia enhance tumor growth via the secretion of pro-tumor and anti-inflammatory factors, especially in GBMs [3] [13] [17]-[22]. Secreted microglial proteins, including stress-inducible protein 1 (STI1), epidermal growth factor (EGF), and membrane type 1-matrix metalloproteinase (MT1-MMP) have been recognized in the involvement of GBM proliferation and migration [18] [22] [23]. Simultaneously, cancer cells release factors that enlist microglial cells and transform their polarization state [24] [25]. A variety of these "chemoattractants" have been thoroughly studied in the glioma-microglia interaction among them are fractalkine (CX3CL1), macrophage chemoattractant protein-1 (MCP1 or CCL2), and versican which functions through toll-like receptor 2 (TLR2) signaling [26] [27].

Recently, immunotherapy has generated significant interest, particularly in its promise to treat various forms of cancer and other diseases (inflammatory, neurological, etc.). Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer cells (NK Cell), cytotoxic T lymphocytes (CTL), etc., work together to defend the body against cancer by targeting abnormal antigens expressed on the surface of tumor cells. Here we used an *in vitro* model to deter-

mine the impact of semiallogeneic vaccines (SAVs) on the modulation of microglia phenotype for the treatment of GBMs.

2. Materials and Methods

2.1. Tissue Culture

Mouse microglial BV2 cells were cultured in RPMI 1640 medium (HycloneTM, GE Healthcare Life Sciences, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. Cells were incubated at 37˚C in a humidified atmosphere in the presence of 5% CO_2. For experiments, each cell line was seeded in six-well plates at a density of 2×10^6 per well 24 h prior to treatment. Cells were stimulated with 50 ng/ml lipopolysaccharide (LPS) combined with 20 ng/ml interferon (IFN)-γ or PBS for 24 h. GL261 cells were a generous gift from Dr. Michael R. Olin (University of Minnesota), and were tested and found free of adventitious agents, before being injected into mice. GL261 cells (H-2b haplotype) were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen), added with 10% of fetal bovine serum (FBS, Atlanta Biologicals). SAV will be generated as described previously [17]. Briefly, RAG cells were combined with GL-261 cells and fused in 50% polyethylene glycol (PEG)-1450; after fusion, the cells were selected in HAT supplemented medium. The surviving RAG × GL-261 semi-allogeneic somatic cell hybrids were isolated and propagated in the selective medium and used in cell-based studies.

2.2. Mouse Microglia Isolation and Culture

Cortices from P0 - P3 C57BL/6 mouse pups were dissected and stripped of meninges and mechanically dissociated with a hand homogenizer and a 25-gauge needle. The cell suspension was seeded into poly-L-lysine-coated (Sigma-Aldrich) T150 tissue culture flasks and maintained in DMEM/F12 with 10% FBS and 1% penicillin-streptomycin for 10 - 14 days to grow a confluent mixed astrocyte/microglia population.

2.3. Mice

C57BL/6 mice were bred and maintained under specific pathogen-free conditions in the Animal Faculty at the University of South Florida. Mice of both sexes aged between 8 and 16 weeks were used. All studies were approved by the Institutional Animal Care and Use Committee at the University of South Florida and the Veterans Affairs Medical Center. This study adhered to the Guide for the Care and Use of Laboratory Animals developed by the Office of Laboratory Animal Welfare.

2.4. Dendritic Cell Preparation

Monocytes were purified by positive sorting using anti-CD14 conjugated magnetic microbeads (Miltenyi Biotec, San Diego, CA). DCs were generated as described by culturing monocytes in RPMI-10% FCS supplemented with granulocyte-macrophage colony-stimulating factor (50 ng/ml; Leukomax, Novartis, Ba-

sel, Switzerland) and IL-4 (1000 U/ml) for 3 - 5 d. Cells were stimulated by the addition of 20 to 100 ng/ml of LPS (Sigma) [28].

2.5. Sorting and Priming of Naive T Cells

Naive CD4+ T cells were sorted by negative selection using the following antibodies: anti-CD45R0 (UCHL-1, IgG2a), anti-CD8 (OKT8, IgG2a), anti-CD16 (B73.1, IgG1), anti-CD14 (144, IgG2b), and anti-CD20 (HB9645, IgG; all from ATCC, Rockville, MD). Purity of the sorted population was 90% - 99% in all the experiments. Contaminating cells were CD4– CD45RA+ CCR7–, most likely natural killer cells. Titration experiments showed that an excess of DCs could bias the response towards TH1, whereas a small number of DCs favored the development of TH2 and nonpolarized T cells. Sorted naive T cells were cultured at a ratio of 10:1 with allogeneic DCs. Cells were then expanded in IL2-containing medium (50 IU/mL).

2.6. Differentiation and Treatment of Monocyte-Derived DCs

Monocytes were isolated by EasySep™ Mouse Custom Enrichment Cocktail (STEMCELL Technologies, France). Recovered monocytes were >90% pure as assessed by CD14 labeling. Monocytes were differentiated to immature DCs during 6 days with 100 ng/ml mouse recombinant GM-CSF (R&D Systems) and 25 ng/ml mouse recombinant IL-4 in RPMI 1640 supplemented with 2 mM glutamine, 10 mM HEPES, 1% penicillin/streptavidin. At day 5, DCs thus differentiated were incubated in complete RPMI 1640 medium supplemented with 10% fetal calf serum. A total of 2×10^6 cells were treated with GL261 cells, RAG cells or SAVs (GL261/RAG hybrid). After another 24 h (day 6), cells were harvested and analyzed. Cell viability was above 90%. T cell-DC co-cultures PBMC were isolated by density gradient centrifugation on Ficoll-Hypaque. CD4+ T cells were purified from PBMC by negative selection using the untouched CD4+ T cell Isolation kit (Miltenyi Biotech, San Diego, CA), according to the manufacturer's instructions. T cell-DC co-cultures were conducted in 96-well flat-bottom culture plates. DCs were treated as described above, collected on day 6, extensively washed, and resuspended in RPMI/10% FCS. 2×10^5 T cells were then co-cultured with DCs in 200 µl of complete culture medium at 10:1 T:DC cells ratio. After 4 days, 50 µl of culture supernatant was frozen for cytokine analysis and T-cells were counted for proliferation.

2.7. Enzyme-Linked Immunosorbent Assay (ELISA)

For the *in vitro* experiment, the media were collected at the indicated time points and stored at −80˚C until use. To measure levels of IL-1β and TNF-α, the media was collected and protease and phosphatase inhibitor cocktail (100× Halt protease and phosphatase inhibitor cocktail, #1861281 ThermoFisher) was added at a volume of 20 µl per 1 ml of media. Supernatant protein concentrations were measured with a BCA Protein Assay Kit (ThermoFisher) according to the manufac-

turer specifications. Levels of mouse IL-1β and TNF-α in media were assayed using high-sensitivity ELISA kits (Quantikine R ELISA, R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer specifications. Briefly, samples were added to the assay plates at a volume of 50 µl/well and incubated for 2 h at room temperature. After washing plates with the wash buffer from the kit, mouse IL-1β and TNF-α conjugates were added to each well and incubated for 2 h. The reaction was stopped and well absorbances were read at 450 nm using a microplate reader. For the *in vitro* experiment, we measured levels of IL-12 and IL-10 in BV2 and RAW 264.7 cell culture supernatants. Briefly, supernatants were collected at the indicated time points after LPS + IFN-γ stimulation and centrifuged at 1200 rpm for 3 min to remove cell debris. The supernatant protein concentrations were measured using a spectrophotometer (NanoDrop R ND-1000, ThermoFisher Scientific Inc., Wilmington, DE, USA) at the A280 absorbance level. Levels of IL-12 and IL-10 in supernatants were assayed using high-sensitivity ELISA kits (Quantikine R ELISA, R&D Systems) according to the manufacturer specifications and the well absorbances were read at 450 nm using an ELISA plate reader.

2.8. Real-Time Polymerase Chain Reaction

For the *in vitro* study, BV2 and primary microglial cells were collected at the indicated time points after LPS + IFN-γ stimulation and RNA was isolated using the same method as that described above. As per the manufacturer instructions, RNA was eluted with 30 - 50 µl of RNase-free H_2O. Samples were immediately aliquoted and stored at −80˚C until use. RNA concentrations were quantified using a spectrophotometer (NanoDrop R ND1000) at the A260 absorbance level. The purity of the RNA was assessed by calculating the A260/A280 ratio of each sample. cDNA was synthesized from 1 µg of total RNA for each sample using the iScript Advanced cDNA Synthesis kit (Biorad). Diluted cDNA was amplified with SYBR R Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in a final reaction volume of 50 µl. The PCR reaction was performed using an MyIQ iCycler (Biorad). The PCR program included initial denaturation at 95˚C for 10 s followed by 40 cycles of 95˚C for 5 s, 60˚C for 34 s, 95˚C for 15 s, 60˚C for 1 min, and 95˚C for 15 s. The nucleotide sequences of the primers used in this study are shown in Table 1. The cycling threshold values for TNF-α, CXCL-10, IL-1β, arginase-1, IL-10, and Ym-1 were normalized to those of β-actin.

2.9. Statistical Analysis

For the study, experiments were repeated 6 times (n = 6) and each sample was a replicate of 3 assays. Data are expressed as the mean ± standard error of the mean (SEM). The groups were compared using a one-way analysis of variance followed by Tukey post hoc tests and unpaired t-tests as appropriate. Differences with a $P < 0.05$ were deemed statistically significant. All statistical analyses were performed using Prism version 5.0 software (GraphPad Software Inc., La Jolla, CA, USA).

Table 1. DNA sequences of the primers for reverse transcriptase polymerase chain reaction.

Mouse cDNA	Primer sequences
TNF-α	Forward, 5'-ACGGCATGGATCTCAAAGAC-3'
	Reverse, 5'-AGATAGCAAATCGGCTGACG-3'
CXCL10	Forward, 5'-GGATGGCTGTCCTAGCTCTG-3'
	Reverse, 5'-TGAGCTAGGGAGGACAAGGA-3'
IL-1β	Forward, 5'-TGTCTTGGCCGAGGACTAAGG-3'
	Reverse, 5'-TGGGCTGGACTGTTTCTAATGC–3'
Arginase-1	Forward, 5'-GAACACGGCAGTGGCTTTAAC-3'
	Reverse, 5'-TGCTTAGCTCTGTCTGCTTTGC-3'
IL-10	Forward, 5'-GCTCTTACTGACTGGCATGAG-3'
	Reverse, 5'-CGCAGCTCTAGGAGCATGTG-3'
Ym-1	Forward, 5'-GGGCATACCTTTATCCTGAG-3'
	Reverse, 5'-CCACTGAAGTCATCCATGTC-3'
β-actin	Forward, 5'-AGAGGGAAATCGTGCGTGAC-3'
	Reverse, 5'-CAATAGTGATGACCTGGCCGT-3'

3. Results

3.1. *In Vitro* Effect of Interferon (IFN)-γ on Microglial Polarization

BV-2 microglial cells were grown in culture as described in the methods section and were treated with 50 ng/ml of lipopolysaccharide (LPS) and with 20 ng/ml of interferon (IFN)-γ. After 24 hours, the media was collected, and the cells were harvested for analysis of M1- and M2-specific markers (**Figure 1(A)**). The control BV-2 cells present with an M2 phenotype as seen in the figure. The cells express Arginase 1 (Arg1), Chitinase 3-like 3 (Ym1), chemokine ligand 2 (CCL2) and mannose receptor C type 1 (CD206) [29]. Alternatively, the cells do not express inducible nitric oxide synthase (iNOS), interleukin-1β (IL-1β), chemokine ligand 5 (CCL5), and chemokine (CXC motif) ligand 1 (CXCL1). Upon stimulation with IFN-γ, the cells switch from the M2 to an M1 phenotype. Likewise, when primary mouse microglial cells are cultured and exposed to both LPS and IFN-γ, they switch from an M2 to M1 phenotype.

3.2. Semiallogeneic Vaccine (SAV)-Induced Maturation of DCs

We investigated the effect of SAVs on the activation of mouse dendritic cells (DCs). Cells were differentiated from mouse peripheral blood monocytes in culture medium. Cells had a typical phenotype of immature DCs at day 6, as defined by high CD11c and low CD14 expression (data not shown). SAV (GL261/RAG hybrid cells), GL261, or RAG cells were added in the cell culture at day 5 for 24 hours. DCs with GL261 or RAG cells did not induce significant modification in

the expression of the analyzed surface markers as compared with control immature DCs. Interestingly, SAV treatment increased the expression of CD80, CD86, CD40, CCR2 and CXCR1 (**Figure 2(A)**). These results indicate that SAV induces a phenotypic maturation of DCs *in vitro* [30]. CCR2 plays a critical role in DC maturation, and CX3CR1 promotes proper development of myeloid precursors into DCs. SAV induced the upregulation of CCR2 and CX3CR1. To characterize the effect of SAV on DC function, we analyzed the production of cytokines and chemokines from DCs (**Figure 2(B)**). SAV induced an increased secretion of TNFα (182 ± 34 pg/mL, n = 6; $^{*}P < 0.01$), interleukin (IL)-1β (235 ± 24 pg/mL, n = 6; $^{*}P < 0.01$) and IL-10 (203 ± 29 pg/mL, n = 6; $^{*}P < 0.01$) compared with GL261 and RAG stimulation. Low quantity of IL-10 could be detected in supernatants of GL261- or RAG-treated DCs and immature DCs (23 ± 9 and 25 ± 7 pg/mL, and 22 ± 14 pg/mL, respectively).

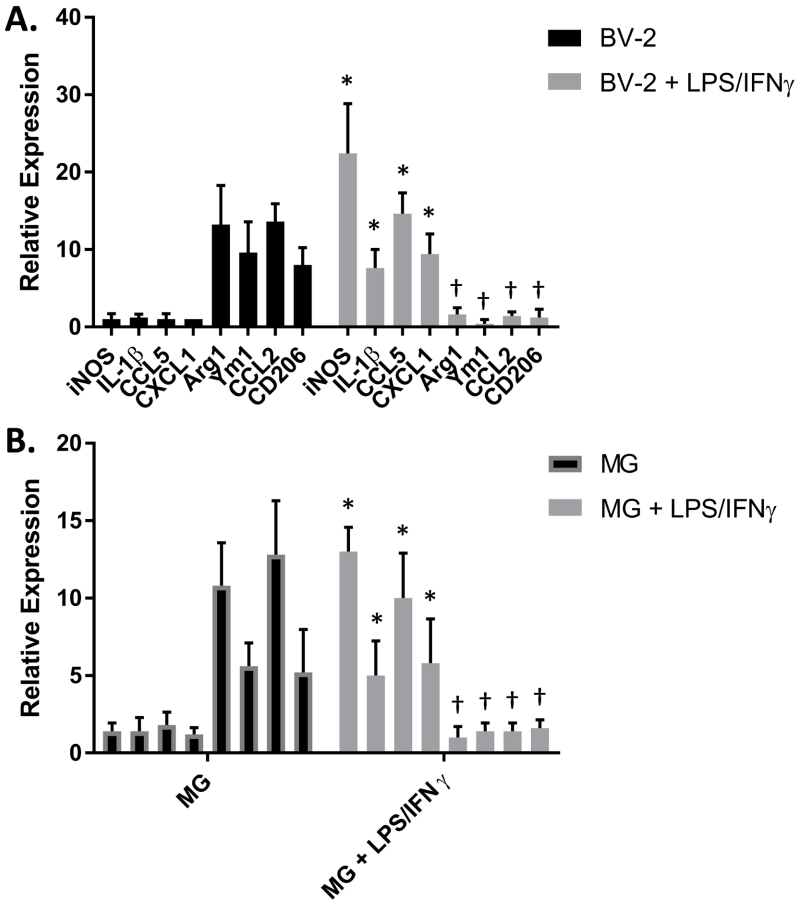

Figure 1. Modulation of microglial phenotype by IFN-γ. (A) BV-2 mouse microglial like cells were grown in culture and treated with 50 ng/ml lipopolysaccharide (LPS) combined with 20 ng·ml interferon (IFN)-γ or PBS for 24 hrs. mRNA expression of selected M1-related and M2-related genes were performed. (B) Primary mouse microglial cells were grown in culture and treated with 50 ng/ml lipopolysaccharide (LPS) combined with 20 ng.ml interferon (IFN)-γ or PBS for 24 hrs. mRNA expression of selected M1-related and M2-related genes were performed. BV-2, untreated cells; BV-2 + LPS/IFN-γ, treated cells. $^{*}P < 0.05$ compared to control cells; $^{\dagger}P < 0.01$ compared to control cells.

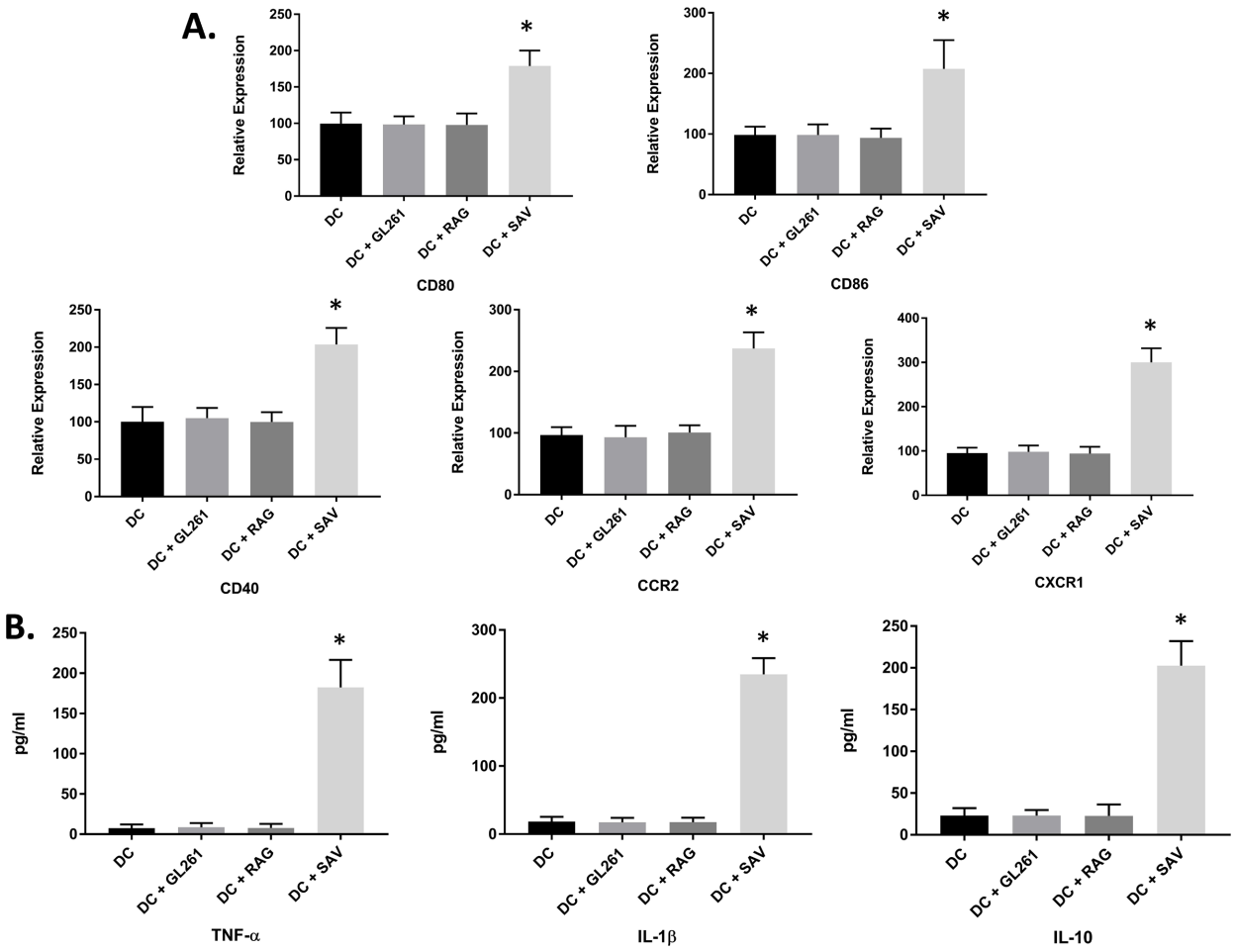

Figure 2. Relative expression of dendritic cell maturation proteins following treatment with cells or vaccine. (A) Primary mouse dendritic cells were treated with GL261 cells, RAG cells or GL261/RAG semiallogeneic vaccine (SAV). The relative expression of CD80, CD86, CD40, CCR2 and CXCR1 were determined using ELISA and western blot analysis. (B) Primary mouse dendritic cells were treated with GL261 cells, RAG cells or GL261/RAG semiallogeneic vaccine (SAV). The concentration of TNF-α, IL-1β, and IL-10 were determined from the media using ELISA analysis. *$P < 0.05$ compared to control cells (DC).

3.3. SAV-Treated DC-Stimulated T-Cell Activation

As mature DCs are of major importance for the stimulation of naive T cells, we compared DCs treated with SAV with DCs, which were treated with GL261 or RAG, in DC-T cell co-culture experiments. We demonstrate that SAV-treated DCs stimulate T-cell proliferation (Figure 3(A)), the addition of SAV during the late stage of monocyte differentiation gave rise directly to mature DCs supporting T-cell stimulation. To study the extent to which the inflammatory mediators SAV polarizes Th-cell development, we analyzed the cytokines secreted by naive T cells in the DC–T-cell cultures. In the co-cultures, SAV-treated DCs induced the release of TGF-β (50 ± 12 pg/mL) and IL-6 (579 ± 52 pg/mL) (Figure 3(B)). T cell stimulated with SAV-treated cells produced interferon (IFN)-γ, whereas no significant IL-4 could be detected (data not shown). Interestingly, a small proportion of T cells produced IL-17 (data not shown). This indicated that SAV may instruct DC to initiate both Th1 and Th17 polarization.

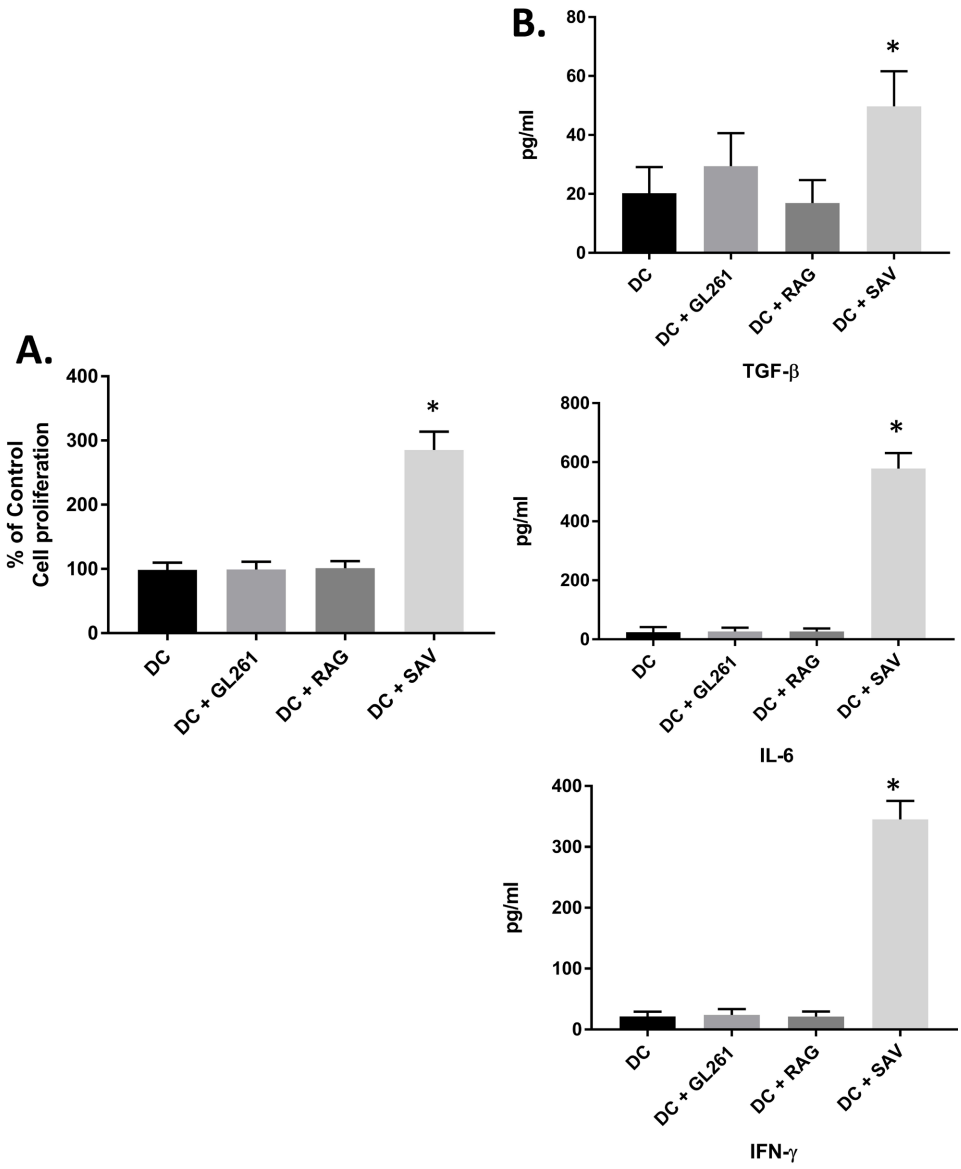

Figure 3. Influence of DCs treated with cells and vaccine on cell growth and cytokine expression. (A) After a 72-hour treatment with cells or vaccine, DCs were washed and co-cultured in triplicate for 5 days with T cells. Proliferative response was evaluated by isolation of T-cells and counting. *$P < 0.05$ compared to control cells (DC). (B) Cytokine section in DC-T0 cell coculture. Culture supernatants were collected after 4 days and level of transforming growth factor-β, interleukin (IL)-6 and interferon (IFN)-γ was determined by ELISA. Results represent the mean ± SEM from 5 independent experiments. *$P < 0.05$, DC + SAV vs DC and others.

3.4. Control of Microglial Polarization by Activated T-Cells

We determined the impact of stimulated T-cells on microglial polarization (**Figure 4**). BV-2 cells alone showed the typical pattern of microglia cells as seen in **Figure 1**. When the BV-2 cells were incubated in the presence of media from the DC cells alone, the pattern of microglial cells was similar to the BV-2 cells alone, *i.e.*, an M2 phenotype. When the BV-2 cells were incubated in the presence of the SAV media (*i.e.*, stimulated T-cells), they showed a conversion from M2 to an M1

phenotype. As seen in the figure, the BV-2 cells in the presence of SAV media showed an increase in iNOS, IL-1β, CCL5 and CXCL1.

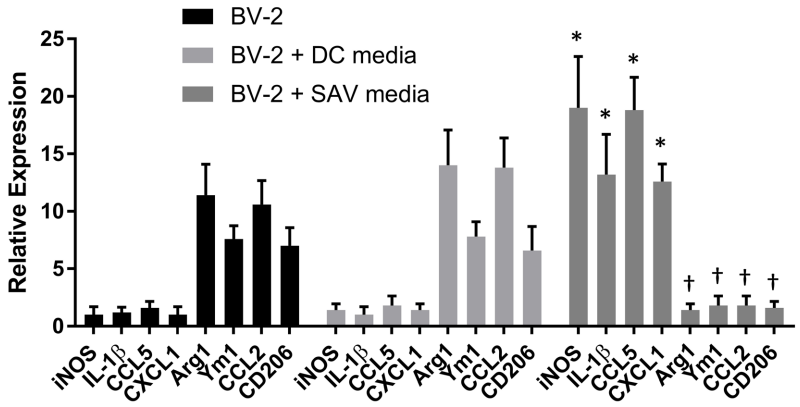

Figure 4. *In vitro* evaluation of the M1 phenotype in BV2 cells after treatment with media from DC-T-cell coculture studies. BV-2 cells were incubated with media from the coculture studies for 24 hours and then mRNA expression of selected M1-related genes in BV-2 cells was determined. RT-PCR was performed on RNA isolated from the cells. β-actin was used for normalization. $^{*}P < 0.05$, compared to the BV-2 media; $^{\dagger}P < 0.01$, compared to the BV-2 and BV-2 + SAV media.

3.5. M1-Polarized Microglia Kill GL261 Tumor Cells

Finally, to determine the impact of the SAV-M1-polarized microglia to the effect on the tumor, the BV-2 cells were included in the presence of the GL-261 cells (**Figure 5**). When the GL261 cells were incubated in the presence of the control media, media derived from the DC cells (DC media) or derived from the SAV cells (SAV media), there was no difference in the cell number (as determined by counting the luciferase positive cells in the culture [31].

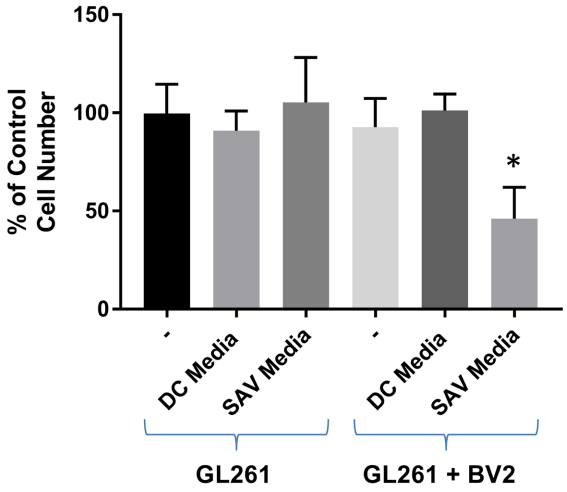

Figure 5. Influence of T-cell mediated responses on microglia activation and GL261 cell number. BV-2 cells were incubated with media from the coculture studies for 48 hours in the presence of the GL261 tumor cells. At the indicated time, the number of GL261 cells was determined by cell counting. $^{*}P < 0.05$, compared to the other treatments.

4. Discussion

Here we report that semiallogeneic vaccines (SAVs) but not the individual cells promote T-cell activation through DCs. This finding is in line with and extends our previous findings where we demonstrated that semiallogeneic vaccines function through the induction of microglial activation to treat glioblastomas (GBMs) [17]. Our studies showed that the presence of microgial cells around the tumor might play an important role in the regulation of tumor formation.

Importantly, this effect on T-cells was mediated by DCs. DCs are antigen-presenting cells (APCs, also known as *accessory cells*) that are part of the mammalian immune system [32]. Their main function is to collect, process foreign antigen material and present it on the cell surface to be recognized by T-cells [33]. They act as messengers between the innate and the adaptive immune systems [34]. Dendritic cells are primarily present in tissues that are in contact with the external environment, such as the skin (where there is a specialized dendritic cell type called the Langerhans cell) and the inner lining of the nose, lungs, stomach and intestines [35]. They can also be found in an immature state in the blood.

Our previous studies have shown that SAVs were effective in the treatment of GBMs. When administered to mice previously injected intracranially with a lethal dose of GL261 glioblastoma cells, mice survival rates were significantly lengthened compared to control animals untreated with SAVs [17]. Tumor volume in the treated mice had significantly decreased compared to the control animals and was the result of a single subcutaneous injection of the vaccine. The data showed that the vaccines had a greater efficacy when used therapeutically rather than preventively.

We have shown that when mice are treated with GL261 to induce GBMs as the tumor grows, microglia are observed adjacent to the tumor cells. However, in the mice receiving the SAVs, the microglia appear to infiltrate the tumor and surround the tumor mass. From these studies, we concluded that microglial cells may be playing an important role in the process of tumor regression or suppression of tumor growth. The data implicates; glioblastoma associated microglial cells may influence growth promoting activity rather than destructive potential [36]. We did not examine the potential for the recruitment of macrophages into the brain that may contribute to the effect. No T-cells were detected in tumors, signifying that they did not play a direct role in the tumor processes. Glioblastoma is an "immunologically cold" tumor, characterized by a highly immunosuppressive microenvironment (TME) with naturally low numbers and dysfunctional T-cells. Semiallogeneic vaccines may overcome this absence of functional T-cells to stimulate a robust response (generation of cytokines) that also targets the patient's own tumor cells [37].

Based on the present results, we hypothesize that SAVs intersect with the immune system signaling axis to provide an opportunity to physiologically shift microglia polarization. However, the therapeutic usefulness of such an approach has to be carefully evaluated *in vivo* studies. We conclude that our data provides func-

tional support for an inflammatory function of SAVs in BV-2 and primary microglial cells. The present *in vitro* study indicates that stimulation of DCs and T-cells with the SAVs signals a process resulting in microglial polarization to an M1 phenotype that could offer new means to modulate the microglia polarization status and treat tumor cells.

5. Conclusion

In conclusion, this study helps to determine the mechanisms of the semiallogeneic vaccines efficacy in the mouse model of glioblastoma. The study demonstrates that activation of microglial cells is critical to the impact of the vaccines and limiting the growth and expansion of the disease process. Overall, the study helps in the translation of the preclinical studies to clinical application by defining the role of the microglia in vaccine treatment.

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Author Contributions

J.Y, and M.S.K. provided the study concept and the design. J.Y., H.Z., and J.K.R. acquired the data. M.S.K. provided the analysis and interpretation of the data. M.S.K. drafted the manuscript. All authors critically reviewed the manuscript for important intellectual content. J.Y. and M.S.K. supervised the study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

ANOVA: analysis of variance; APC: antigen producing cells; CTL: cytotoxic T lymphocytes; DC: dendritic cells; EGF: epidermal growth factor; GBM: glioblastoma multiforme; LPS: lipopolysaccharide; MHC: major histocompatibility complex; MMP: matrix metalloproteinases; NK: natural killer cells; PBMC: peripheral blood mononuclear cells; SAV: semiallogeneic vaccine