Review Article

Chronic inflammation drives glioma growth: cellular and molecular factors responsible for an immunosuppressive microenvironment

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ABSTRACT

This review examines glioma disease initiation, promotion, and progression with a focus on the cell types present within the tumor mass and the molecules responsible for the immunosuppressive microenvironment that are present at each step of the disease. The cell types and molecules present also correlate with the grade of malignancy. An overall "type 2" chronic inflammatory microenvironment develops that facilitates glioma promotion and contributes to the neo-vascularization characteristic of gliomas. An immunosuppressive microenvironment shields the tumor mass from clearance by the patient's own immune system. Here, we provide suggestions to deal with a chronically-inflamed tumor microenvironment and provide recommendations to help optimize adjuvant immune- and gene therapies currently offered to glioma patients.

Key words: Astrocytoma, glioma, immunotherapy, inflammation, microenvironment

INTRODUCTION

The World Health Organization (WHO) grade IV glioma is called glioblastoma, and is formerly termed glioblastoma multiforme (GBM) because its appearance can take on a variety of morphologic forms. GBM is the most common and lethal of all primary malignant brain tumors, and is responsible for over 13,000 deaths per year in the United States.[1] The median survival is approximately 15 months.[2] The first line of treatment for this disease is usually surgical resection followed by concurrent chemo-radiotherapy. Importantly, the invasive tumor cells that remain after surgery and survive these aggressive treatments are likely responsible for tumor recurrence. Thus, alternative novel therapies that enhance or work in conjunction with conventional treatments are being actively pursued. While immunotherapies seemingly provide viable, theoretically effective options, in practice they have produced mixed results. The glioma microenvironment is highly immunosuppressive, thereby inhibiting the efficacy of immune treatments. Microenvironmental factors allow glioma cell evasion from the immune system. The source of the factors is not solely derived from the tumor mass, but rather is also a consequence of chronic inflammation present in the tumor microenvironment.

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GLIOMAS AND CHRONIC INFLAMMATION

Chronic inflammation can influence a wide range of ailments including heart disease, stroke, Crohn's disease, rheumatoid arthritis, multiple sclerosis, asthma, Alzheimer's, depression, fatigue, neuropathic pain, and - relevant to our discussion - cancer.[3-13] Indeed, it is thought that around 15% of all cancer-related deaths are in some form linked with inflammation as a result of bacterial or viral infections.[14] Further, chronic inflammation occurring within the microenvironment of tumor lesions is now thought to either drive the first malignant-conferring genetic mutations and/or induce them as a result of oncogene expression.[14]

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GBM TYPES AND STAGES IN DISEASE PROGRESSION

GBM is classified as one of two types: primary or secondary GBM. Primary GBM arises as the *de novo* high grade disease that has no discernible stages of progression. Secondary GBM, on the other hand, arises as low grade and over time progresses to a higher-grade of malignancy. Therefore it is extremely difficult - if not impossible - to analyze the changes that arise in a step-wise manner for primary GBM, while the progression of secondary GBM can be closely followed. However, recent genomic analysis of primary resected GBM tissue has allowed for a second dimension of their grouping by gene expression/mutations patterns: neural, pro-neural, classical, and mesenchymal.[15]

Contemporary theory links the phenotypic characteristics observed in the tumor microenvironment to each of three stages of glioma pathology: initiation, promotion and progression.[16] A defect in the switch for wound repair may play a major role in glioma development because it leads to "type 2" chronic inflammation that fails to shut off and this may drive gliomagenesis. Further, "type 2" chronic inflammation, which is propagated indefinitely in the tumor microenvironment, may be critical in triggering tumor initiation. The effect of chronic inflammation that develops in the tumor microenvironment is far reaching beyond the initiating effects, and it may also drive the second stage of disease, glioma promotion. The third and last stage of the disease, glioma progression, is a stage of the disease that loops back adding to the intensity of the underlying inflammation.

The initiating event

Embracing the cell of origin model, the theoretical consideration of the inflammatory-initiating event beginning with a specific mutation in the cancer stem cell may occur either through oncogene over-expression/stimulation or production of an inflammatory onco-metabolite. A traumatic event at a specific site in the brain may activate all the necessary signal transduction pathways to initiate inflammation. The site of injury could be a source of micro-damage induced by chronic stress, depression, or some other factor extrinsic to the host.[17]

As another consideration, the source of the micro-damage and neural-degeneration may be the cancer stem cell itself. Recently, a novel genetic mutation encoding the isocitrate dehydrogenase 1 (IDH1) protein known to be present in a large number of low-grade to secondary GBM tumors has been identified as one of the possible first events in disease initiation.[18] Subsequently, following the identification of this mutated oncogene, its function was revealed. Cells encoding the mutant IDH1 protein were found to convert, in an irreversible reaction, α-ketoglutarate to 2-hydroxyglutarate (2-HG).[19] The function of the accumulation of 2-HG and its role in disease progression is not yet fully elucidated. The fundamental role of 2-HG has been difficult to assign partly because its role is unlike conventional onco-metabolites characterized thus far. Rather than promoting disease progression by conferring itself with a proliferative or selective advantage, 2-HG initiates development of the cancer stem cell niche within the frontal lobe. Thus, the onco-metabolite 2-HG is the first example demonstrating that although the derivation of cancer is mono-cellular in nature at initiation, much like tumor promotion and progression, complicated heterogeneous interactions involving multiple cell types occurs. The cellular interplay also offers an explanation to the challenges associated with establishing a tumor model containing solely the IDH1 mutation both *in vivo* and *in vitro*.[20] This cancer stem cell niche may in turn provide the mutant IDH1 stem-like cell with the necessary factors to promote self-renewal, further genetic mutations, and ultimately disease progression. Indeed, previous studies in patients suffering from the genetic disorder known as D-2-hydroxyglutaric aciduria, where the accumulation of 2-HG is observed, show ROS-mediated neural excito-toxicity upon NMDA receptor chronic potentiation by 2-HG.[21,22] The neural excito-toxicity fueled by the $IDH1^{mut}$ stem-like cell may initiate a neural-inflammatory cycle of wound repair ultimately leading to pro-creation of a cancer stem cell niche that promotes glioma formation and immune evasion. This niche provides the cancer stem cell with growth factors to sustain proliferation and an environment that promotes the emergence of more genetic mutations. Indeed, a connection between excitotoxicity and inflammation has been proposed to be linked by interleukin-1β.[23]

Tumor promotion

Inflammation is the first line of defense in response to tissue injury and/or infection. Pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, Interleukin (IL)-1β, and IL-6 are synthesized to initiate the inflammatory cascade.[24] IL-1 has been shown to be a key mediator in the proliferation of "reactive astrocytes".[25] Next, either of two types of inflammatory processes may be activated depending on the stimulus. In the presence of microbial infection or necrotic cell death classical "type 1" inflammation ensues, characterized by the appearance of activated T helper (Th) 1 lymphocytes.[26] In the presence of parasites, allergens, or phosphatidylserine positive early apoptotic cells the Th2 inflammatory cascade is activated. Interestingly, Th2 inflammation is closely related to wound repair. Indeed, the principal mode of

action against helminth infection is the "walling off" of large bodies through granuloma formation which bears resemblance to the glial scar encountered in central nervous system (CNS) repair.[27]

The initiation of the classical inflammatory response is marked by the localization and subsequent activation of blood circulating monocytes into M1 macrophage. The M1 macrophage are activated by cytokines produced by Th1 cells, like interferon-γ (IFN-γ), TNF-α, or after recognition of pathogen-associated molecular pattern molecules, through toll-like receptors (TLRs) or C-type lectin receptors. Upon activation, the M1 macrophage promote a proinflammatory environment by releasing cytokines such as TNF-α, IL-1, IL-6, IL-12, IFN-γ, and IL-23. IL-12 stimulates IFN-γ production in T lymphocytes and natural killer (NK) cells.[28] Phenotypically, the M1 phenotype is associated with cell mediated cytotoxicity, tissue injury and destruction. Thus, the presence of the M1 macrophage is counter-productive once the invading threat is neutralized and tissue repair is in order. The resolution of the inflammatory response and transition into wound repair is facilitated by the M2 macrophage. One of the key events leading to immunosuppression and activation of "type 2" inflammation is apoptotic cell death of recruited neutrophils.[26] The apoptotic neutrophils signal to close classical inflammation and thus modulate immunosuppression after their engulfment by macrophages. In response, the macrophage upregulate expression of the Th2 anti-inflammatory cytokine IL-10, while significantly downregulating the pro-inflammatory cytokines TNF-α, IL-1β, and IL-12.[29]

Several subtypes of the M2 macrophage exist depending on the inflammatory program that is activated and required.[30] The M2a or alternative macrophage is activated by the cytokines IL-4 and IL-13, and these macrophages are specialized to carry out the allergic response and the killing and encapsulation of parasites. The M2b macrophage is activated by ligation of TLRs + immune complexes and the IL-1 receptor. This macrophage subset is primarily responsible for immune regulation and activation of the Th2 program. The M2c macrophage, activated by the cytokine IL-10, is primarily responsible for matrix deposition and tissue remodeling. Recently, a fourth and distinct subtype, termed the M2d subset, has been identified. This subset is activated by IL-6 and is thought to aid in tumor metastasis and progression.[31] The primary cells responsible for the synthesis of those cytokines are eosinophils, basophils, and $CD4^+$ Th2 cells, and tumor cells.[32,33] M2 macrophage down-modulate the release of IL-1, IFN-γ, IL-12, and TNF-α.[34,35]

Also, the recruitment and/or activation of T regulatory lymphocytes are thought to play a key mediating role in the "type 2" inflammatory process.[36-38] Indeed, regulatory T cells have been found not only to be present in the peripheral circulation of glioma patients in increased percentages compared to controls, but also to infiltrate glioma tissue in a tumor grade-dependent manner.[39,40] Interestingly, some encouraging anti-tumor responses have been obtained in attempts to neutralize the substantial peripheral regulatory T cell populations encountered in glioma patients with systemic administration of TLR ligands.[41,42] For example, systemic administration of a TLR9 ligand enhanced survival, decreased the number of peripheral regulatory T cells and enhanced the antigen-presenting capacity of infiltrating microglia.

Recently, there have been many studies documenting a decreased risk of glioma development in individuals with asthma, which is also thought to be driven by "type 2" inflammation.[43,44] Reduced immunoglobulin E levels have been found in patients who developed glioma. Further, additional studies have found that specific polymorphisms in genes encoding IL-4RA and IL-13, both factors that induce IgE production in immune cells, are found to be inversely correlated with glioma development. This apparent contradiction can be reconciled by considering the macrophage subtype that predominates in each pathology. The M2a macrophage are induced by IL-4 and IL-13, express Fc-epsilon receptors, and are involved in the allergic response. On the other hand, the M2b macrophage are induced by engagement of the IL-1 receptor and/or ligation of TLR +/- immune complexes, they express Fc-gamma receptors and are involved in immune regulation.[45,46] It appears that patients developing asthma, as a result of hyperactive IL-13 or IL-4 receptor signaling, are at lower risk of developing gliomas; this may be due to the preferential activation of the M2a subset, which may not be as advantageous to the developing glioma mass that is dominantly populated by the M2b-d macrophage subtypes. IL-10, damage associated molecular pattern molecules, and IL-6 are highly expressed in GBM tissue, where they localize to the macrophage/microglia population.[31,47-51] Further, it has been shown that the presence of IL-4 or IL-13 inhibit the proliferation of astrocytes and low-grade astrocytomas, but not GBM.[52]

In glioma tissue, macrophages/microglia can account for up to 30% of the total lymphocytic infiltrate present in the tumor mass.[53,54] It is now accepted that the macrophage and microglia populations found within glioma originate from distinct progenitor cell populations. Infiltrating macrophages are derived from the bone marrow, whereas microglia are brain-resident;

they originate from primitive progenitors in the yolk sac and migrate into the CNS during early embryo development (days 8.5 to 9.5).[55,56] It has also been clearly demonstrated, using parabiosis (a technique that surgically connects the circulatory system of two organisms) and experimental auto-immune encephalomyelitis models, that circulating monocytes do not invade the CNS unless the CNS is preconditioned with irradiation or the blood-brain barrier is compromised/damaged.[57-60] Interestingly, a key distinction between "type 1" and "type 2" inflammation is that the latter activates bone-marrow derived macrophage in the CNS and/or brain resident microglia.[61,62] Taken altogether, microglia are probably recruited to the glioma microenvironment at all stages of malignancy, whereas a majority of the macrophages accumulate only after insult or blood-brain barrier breakdown, when chronic "type 2" inflammation is dominant in the glioma microenvironment.

Convertibility of macrophage from an M1 to an M2 polarized state is driven by factors produced by the local glioma microenvironment. Indeed, secreted or displayed glioma factors are capable of manipulating macrophage and microglial behavior that favor tumor survival and growth. Resting microglia are characterized by a ramified morphology; they display extensive branched projections that aid in continuous surveillance of the CNS microenvironment.[63] Glioma cells secrete key immunomodulatory factors that suppress "type 1" immune activity, such as IL-10, IL-4, IL-6, transforming growth factor (TGF)-β, and prostaglandin E2.[64-66] The cytokines IL-10, IL-4, and IL-6 have been shown to induce an M2 rounded morphology that is typical of activated microglia, whereas the T helper (Th) 3 cytokine, TGF-β, is known to inhibit microglial cell proliferation and the expression of pro-inflammatory cytokines *in vitro*.[67] Due to the dominant effect that glioma cells and their secreted factors have on the surrounding cells, it is likely that glioma-recruited microglia preferentially adopt an M2 phenotype. Studies that delineate the interactions between glioma cells and macrophages/microglia are still warranted.

Inflammation status temporally may play a pivotal role in cancer development. The "type 1" pro-inflammatory process cannot be sustained in the absence of proper stimulation. In brain trauma, "type 1" monocyte recruitment from the blood becomes negligible over time, but in low grade gliomas, constant neuronal damage from continuous 2-HG expression may prevent the Th1 inflammatory process from subsiding. Eventually both "type 1" and "type 2" immune responses are both activated leading to chronic inflammation. The strength of "type 2" *vs.* "type 1" inflammation, which is generally reflected by the serum Th2/Th1 cytokine ratio(s), has been positively correlated with the grade of glioma malignancy.[68] As another example, patients displaying genetic polymorphisms of the IL-1, IL-10, and TNF-α genes are at higher risk for developing gastric cancers.[69,70] Studies with human glioma tissues and patient sera indicate Th1, Th2, and Th3 cytokine deregulation as evidenced by increased Th2 associated cytokines such as IL-10 and the Th3 associated cytokine TGF-β. This increase is offset by a concomitant decrease in Th1 cytokines such as IL-12, IFN-γ, TNF-α, IL-2, and many of their corresponding receptors.[71,72]

Tumor progression and invasion

Cancer cells become "self-sufficient" once they have accumulated the proper genetic mutations to support their own growth. Some of the key findings associated with this stage in disease development include independence from external growth factors, the ability to bypass cell senescence, and dysfunctional apoptotic pathways. In order to develop glioma subtypes, two combinations of genetic mutation may prevail that involve the mutation of the IDH1 gene and p53, resulting in astrocytoma formation, or 1p/19q loss of heterozygosity (LOH) leading to formation of oligoastrocytomas or oligodendrogliomas.[73] Such mutations increase the proliferative rate of cancer stem cells, which allows them to grow outside of their niche. This concept was confirmed in studies using an IDH1 mutant model both *in vitro* and *in vivo*.[74] Tumor samples derived from WHO grade II and III gliomas were successful in retaining the mutation in neurosphere culture. The lower grade gliomas proliferate slowly and are difficult to utilize in standard *in vivo* xenograft models.

The late stages of "type 2" inflammation primarily consist of extracellular matrix deposition, angiogenesis, and tissue remodeling. Once gliomas becomes "self sufficient", these late stage processes are aberrantly used by the proliferating glioma mass to fuel and sustain proliferation. In particular, myeloid derived suppressors cells are now thought to play a large role in facilitating glioma angiogenesis, neo-vascularization, and invasion [Figure 1].[75] Recent studies have shown that the Tie2-expressing monocyte population is pro-angiogenic, expressing relevant gene transcripts [e.g. matrix metalloproteinase 9, vascular endothelial growth factor (VEGF), cyclooxygenase 2, and wingless-type MMTV integration site family, member 5A] necessary for angiogenesis and neo-vascularization.[76,77] Some myeloid derived suppressor cells also seem to contribute to the integrity of neo-endothelium of tumor vessels because they express endothelial markers, such as CD31 and VEGF receptor and can morphologically resemble endothelial cells.[78] Microglia also localize near the invasive border of the glioma mass at three-fold higher

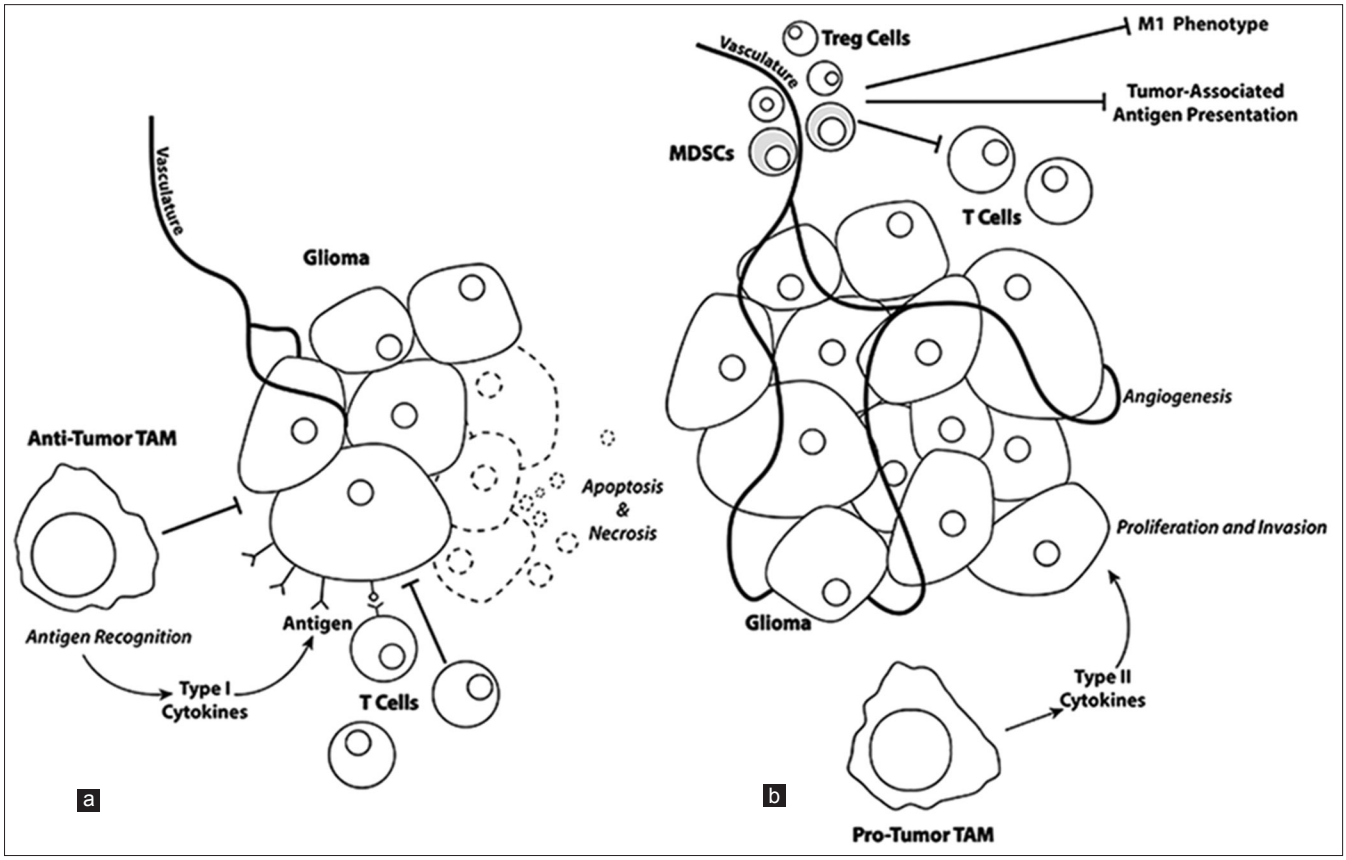

Figure 1: (a) Glioma cell proliferation and invasion is negatively affected when T cells recognize tumor-associated antigens resulting in recognition and tumor cell injury that reduces the tumor mass. (b) Mobilization of T regulatory (Treg) cells and myeloid-derived suppressor cells (MDSCs) to the tumor mass, as well as changes in the phenotypes of tumor-associated macrophages (TAM) result in pro-tumorigenic regulation with increases in tumor cell proliferation, angiogenesis, and invasion

numbers than tumor associated macrophages, suggesting that they might play a key role in glioma invasion.[79]

Recent genetic microarray analyses of glioma patient tumors have revealed variations between glioma subtype progression, invasion, and response to therapy. In patients enrolled in a phase I dendritic cell (DC) vaccine therapy clinical trial, we identified significant trends in the mesenchymal glioma subtype, including its progression and its particular responsiveness to treatment.[80] It may be worth exploring if the mesenchymal subgroup of GBM patients have tumor cells carrying LOH in the neurofibromatosis-1 (NF-1) gene, also have NF-1 heterozygous microglia populating the GBM tumor microenvironment. NF-1 heterozygous microglia are essential in driving optic nerve astroglioma with NF-1 LOH.[81] Further, NF-1 heterozygous microglia drive optic nerve glioma by facilitating a relatively more "type 1" chronic inflammatory microenvironment through increased c-Jun-NH_2-kinase (JNK) signaling leading to the constitutive expression of higher levels of pro-inflammatory cytokines and proteins TNF-α, IL-1, iNOS, and Cox2.[82] The JNK and ERK1/2 pathway is not only responsible for the expression of pro-inflammatory cytokines, but also for the repression of the transcriptional potential of Smad3 activated by TGF-β as well.[83-85] Conversely, TGF-β1 mediates its effects through inhibition of the ERK pathway.[86] Among its many effects, TGF-β1 in the tumor microenvironment is an important regulator of glioma invasion.[87,88] The overactive JNK signaling in NF-1 heterozygous microglia may lead to a constitutively active state of microglia based on morphology and expression profiles. The existence of activated macrophage/microglia within the GBM tumor mass may facilitate a relatively more favorable immunogenic microenvironment that maintains T cell activation once they are mobilized to tumor by DC vaccination. This theory underscores the crucial role that microglia may play in the tumor microenvironment by potentiating the immune responses against tumor cells. Indeed, it has been proposed that modified microglia may have benefit for glioma treatment.[89,90]

Indeed, modulating the microglia in the tumor microenvironment of wild type NF-1 patients may prove to be an important aspect to glioma therapy. IL-10-mediated inhibition of NF-κB heterodimer (p50/p65) formation leads to an over-expression of the NF-κB homodimer (p50/p50), which prevents transcription elongation of various genes encoding pro-inflammatory cytokines. This is predominantly responsible for the tolerant M2 macrophage phenotype encountered in the microenvironment of wild type NF-1 patients.[91-93] Interestingly, IL-10 is a cytokine translated in tandem with other pro-inflammatory

cytokines in response to lipopolysaccharide stimuli.[94] This attribute is most likely an evolutionarily hard-wired negative feedback mechanism to preserve the cyclic response curve characteristic in NF-κB signaling.[95] We propose that the presence of IL-10 in the glioma microenvironment substantially dampens the transient pro-inflammatory activating pulse delivered by tumor-lysate activated DCs and booster injection of TLR agonist. This mechanism is circumvented in NF-1 heterozygous microglia through deregulated Ras/Rac1/JNK/c-Jun/AP-1 signaling, which operates in parallel and independent of the NF-κB signaling pathway [Figure 2]. Deactivating antibodies against IL-10 may restore the formation of the NF-κB heterodimer ultimately leading to a M1 microglia phenotype without overshooting the pro-inflammatory response, which may have detrimental effects on patients. Then, effectors cells mobilized by the vaccine can operate and maintain functionality by encountering a skewed microenvironment to a "type 1" pro-inflammatory state. Ultimately tumor regression may lead to a natural resolution of the inflammatory phase mediated in large part by IL-10.

IMMUNE AND GENE THERAPEUTICS THAT ENGENDER INFLAMMATION

Our translational immunotherapy research team has a long-standing interest in the development of novel therapeutic options for brain tumor patients. Our group and others have preclinically explored active and passive immune and gene therapy approaches, some of which are translated to the clinic.[96,97] The therapies are generally designed as adjuvant treatments and entail tumor resection followed by administration

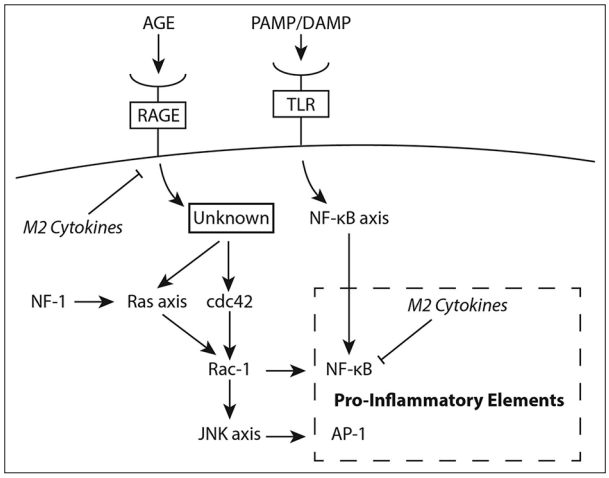

Figure 2: Signaling imparted through interactions of advanced glycation end products (AGE) with the receptors for AGE (RAGE) versus interactions of pathogen associated molecular pattern (PAMP) or damage associated molecular pattern (DAMP) molecules with Toll like receptors (TLR). The schematic shows the two signaling pathways that operate in parallel but independent to each other; both lead to expression of pro-inflammatory cytokines. The presence of anti-inflammatory (M2) cytokines inhibits the expression of pro-inflammatory cytokines of both inflammatory response pathways. NF: Neurofibromatosis; JNK: Jun-NH2-kinase

of the experimental agent. Surgical resection serves multiple purposes. Importantly, resection reduces tumor burden and the immunosuppressive factors present in the tumor microenvironment that will enhance the effectiveness of the immunotherapy. Also, the degree of the mobilized inflammatory response is minimized. The tumor specimens are valuable since they serve as a source of tumor associated antigens to make vaccines. Likewise, tumor specimens can be processed and placed into culture where the cells can serve in *in vitro* studies and as target cells for cytotoxicity testing.[98]

We have successfully used tumor-lysate pulsed DC vaccines that are given with or without TLR agonist; they represent an active immunotherapy strategy designed to enhance cell-mediated immunity.[99] The conclusion of a phase II clinical trial has shown the vaccine treatment to extend median survival to 34 months. It appears that the treatment has a relevant role in flagging the tumor cells remaining after surgical resection.[100] We have also examined a passive immunotherapy approach that utilizes effector alloreactive cytotoxic T lymphocytes (alloCTL) that are intratumorally implanted with low doses of Interleukin-2.[101] The allogeneic CTL are trained *in vitro* to target patient human leukocyte antigens that are present on glioma cells but not on normal neuroglia. A pilot clinical study described at www.clinicaltrials.gov (NCT00068510),[102,103] suggested a clinical response in recurrent WHO grade III gliomas. These studies led to a second phase I dose escalation trial that is currently open for patient enrollment at University of California, Los Angeles (www.clinicaltrials.gov; NCT01144247).

In another gene therapy approach, transduction of glioma cells with retroviral replicating vectors (RRV) coding for pro-drug activating enzymes followed by their exposure to non-toxic pro-drug has also proven to be another potent cancer therapy strategy. Non-cytolytic RRV are particularly well suited for the treatment of primary or metastatic brain tumors. In the CNS, normal brain neuroglial cells are relatively quiescent, thus, the dividing glioma cells are selectively targeted by the RRV. After achieving genomic integration, the viral constructs can stably seed the tumor mass and replicate within the tumor cells even as they infiltrate *in vivo*. Pro-drug administration results in targeted destruction of the cells harboring the RRV. Such an approach utilizes RRV coding for yeast cytosine deaminase. Upon administration of the pro-drug, 5-fluorocytosine, the drug is converted to its toxic form, 5-fluorouracil. If sufficient time is allowed for RRV spreading, the administered prodrug converts to a cytotoxic form, killing infected cells and providing tumor cytoreduction. Predicated upon successful and extensive preclinical testing,[104-108] phase I clinical trials

are testing the RRV suicide gene therapy in recurrent glioma patients (www.clinicaltrials.gov, NCT01156584; NCT01470794; NCT01985256).

Most recently, our attention has turned to preclinical studies examining a more aggressive combined immunogene therapy approach. RRV-transduced alloCTL have effector and delivery functions. If combined with pro-drug administration, the immunogene therapy is more efficacious *in vivo* than the individual therapies and control groups. Better extension was obtained in the survival of mice bearing orthotopic intracranial implants of breast carcinoma.[109,110] The immunogene therapy is similarly being tested in a syngeneic mouse glioma model. If the data look as promising in this model after optimizing doses and timing, combining the therapies should be easily translatable since both are being individually tested now in the clinic.

Challenges in immunotherapy

Immunotherapeutics do not always robustly provide efficacious treatment for gliomas. This may be due to the concurrent activation of both pro- and anti-inflammatory responses and this may have clinical and therapeutic consequences [Figure 3]. Clinically, immunotherapy entails protracted treatments. While manageable in theory, maintaining patients on immune treatments over the extended period necessary to effect a cell-mediated immune response has proven difficult.[102] Furthermore, inflammation associated with immune therapy is indistinguishable from tumor progression on follow-up magnetic resonance images; a clinician must give benefit of doubt and recommend other treatments inhibiting possible tumor growth. The immunotherapy is unfortunately either interrupted or incompletely tested. With the inability to distinguish pseudo- from tumor- progression, completion of trials is difficult, especially with the availability of drugs such as Avastin, Temodar, or other chemo- or radio-therapeutics for use at recurrence. Developing an appropriate set of neuroimaging parameters to distinguish inflammation from tumor growth would help advance this field. Perhaps one solution would be to offer immunotherapy upfront, or integrate it with standard of care treatments.

Therapeutically, the chronic inflammation that develops and worsens in correlation with glioma grade promotes a skewed "type 2" inflammatory state, both in the local tumor microenvironment and systemically.[40] Once gliomas are in the progression phase (i.e. pro-wound repair) deactivation of T cell-mediated immune response occurs. To effectively mount a host-generated, anti-tumor response immune homeostasis must be "reset" and skewed towards a "type 1" inflammatory state. An interesting possibility to generate a (Type 1) inflammatory response is the administration of attenuated microbes. Indeed, Bacillus Calmette Guérin (BCG) is effectively used for immunotherapy of superficial bladder cancer.[111] The success of BCG as a therapeutic modality for low-grade bladder cancer can be effectively attributed to two characteristics: immunogenicity and anti-tumor targeting. In BCG tumor models, the initial presence of both Th1 and Th2 inflammatory cytokines was also observed, but then later skewed towards Th1 cytokines that in particular involved the up-regulation of IFN-γ.[112] However, the situation is complicated for the treatment of high-grade glioma. Studies of immunosuppression have shown that once "type 2" inflammation has been activated, challenge with a bacterial lipopolysaccharides fails to skew the cytokine expression towards "type 1" in a time-dependent manner.[29] Thrombospondin receptor (CD36) expressed on macrophages among other cell types formed a "molecular bridge" between anionic sites on apoptotic cells and CD36. This cell-cell signaling interaction was sufficient to signal the resolution of inflammation and activation of "type 2" inflammation. Further, antibodies against thrombospondin prevented its binding to CD36 receptor leading to a decrease of IL-10 and restored TNF-α, IL-1β, and IL-12 in the presence of apoptotic cells. Thus, it appears that immune homeostasis must first be restored for high-grade tumors that are driven by "type 2" inflammation before further intervention

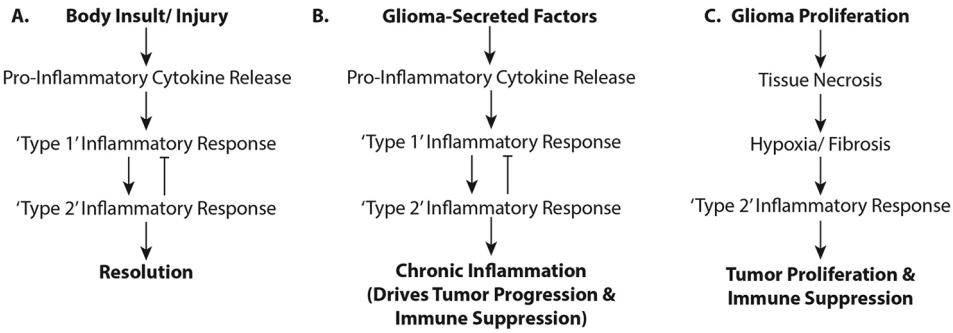

Figure 3: Activation of pro- and anti-inflammatory responses in glioma patients. The flowcharts illustrate (a) the "normal" physiologic processes in the inflammatory response and its resolution; (b) the physiologic processes occurring when glioma-secreted factors influence a state of chronic inflammation resulting in glioma progression; and (c) how rapid glioma growth creates a necrotic/hypoxic environment supporting tumor proliferation and immunosuppression

to activate the "type 1" inflammatory response can be implemented. Enhancing the endogenous immune response by deactivating the ensuing chronic inflammatory tumor microenvironment might provoke an immune response potent enough to activate and mobilize endogenous CTL and NK cells to eliminate the threat posed by high-grade cancerous masses.

CONCLUSION

It has long been held that tumor cells outwit the host's defenses by altering their own cellular signaling pathways. The pathway exploited to achieve malignancy may be a combination of unique derivations. Gliomas are known to exhibit compensatory activity in that when supplied with selective pressure from one treatment, they readily adapt with other mutations to survive. Other mounting evidence now suggests that some of the pathways exploited by cancer cells adopt a more malignant phenotype and are simply responses to the stimuli created by the rapidly dividing tumor cells rather than novel re-circuited pathways exploited by neoplastic cells for growth. One of the crucial responses facilitating and nurturing cancerous transformation is inflammation. A chronically active inflammatory microenvironment provides the developing cancerous mass with proliferative and mutational factors necessary to realize "self-sufficiency". It is evident that some tumors can bypass this "nurturing stage" as might be expected with primary GBM. Regardless, once this "self-sufficiency" is realized, the tumor is able to survive outside of the cancer stem cell niche. Empowered with constant proliferative cues the tumor mass divides uncontrolled. The increased proliferation results in necrosis and the resultant environment is skewed more strongly towards the Th2 inflammatory response. Thus, for high-grade gliomas a higher Th2/Th1 cytokine ratio supports the production of other immunosuppressive factors. To mount a successful cytotoxic anti-tumor response, it is crucial to restore a balanced Th2/Th1 cytokine ratio of 1:1 or less. This should decrease the proliferative rate of the tumor mass as well, since it is the Th2 response that ultimately works with the tumor cell to drive the angiogenic response. Ultimately, successful brain tumor immunotherapy should leave patients with intact immunosurveillance function and the ability to enact a cell-mediated response in the event of recurrence.

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