Neuro-Oncology Advances

6(1), 1–12, 2024 | https://doi.org/10.1093/noajnl/vdae012 | Advance Access date 25 January 2024

Potential roles for efferocytosis in glioblastoma immune evasion

Ian A.J. Lorimer

Cancer Research Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada (I.A.J.L.); Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada (I.A.J.L.); Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Ontario, Canada (I.A.J.L.)

Corresponding Author: Ian A.J. Lorimer, PhD, Cancer Research Program, Ottawa Hospital Research Institute, 501 Smyth Road, Ottawa K1H 8L6, Canada (ilorimer@ohri.ca).

Abstract

Glioblastoma is an aggressive and incurable brain cancer. This cancer establishes both local and systemic immunosuppression that creates a major obstacle to effective immunotherapies. Many studies point to tumor-resident myeloid cells (primarily microglia and macrophages) as key mediators of this immunosuppression. Myeloid cells exhibit a high level of plasticity with respect to their phenotype and are capable of both stimulating and repressing immune responses. How glioblastomas recruit myeloid cells and exploit them to avoid the immune system is an active area of research. Macrophages can acquire an immunosuppressive phenotype as a consequence of exposure to cytokines such as TGFB1 or IL4; in addition, macrophages can acquire an immunosuppressive phenotype as a consequence of the engulfment of apoptotic cells, a process referred to as efferocytosis. There is substantial evidence that glioblastoma cells are able to secrete cytokines and other factors that induce an immunosuppressive phenotype in macrophages and microglia. However, less is known about the contribution of efferocytosis to immunosuppression in glioblastoma. Here I review the literature in this area and discuss the potential of efferocytosis inhibition to improve glioblastoma response to immunotherapy.

Key Points

- Macrophages are key mediators of the immunosuppressive environment in glioblastoma.
- Efferocytosis contributes to the immunosuppressive phenotype of macrophages in glioblastoma.

Glioblastoma

Glioblastoma is the most common type of brain cancer in adults. Standard treatment consists of surgery followed by radiation and temozolomide chemotherapy. While these treatments improve survival, the improvements are small, and median survival is still only approximately 16 months, with a 5 year survival rate of <5%.[1] Targeted therapies that have shown benefit in other cancer types (eg, tyrosine kinase inhibitors) have generally not been effective in glioblastoma. This is thought to be a consequence of the extensive heterogeneity of this cancer. Similarly, immune checkpoint inhibitors, while very beneficial to subsets of patients with melanoma and several other cancers, showed no benefit when tested in large, randomized trials of glioblastoma patients.[2] A possible exception to this is a small trial in which glioblastoma patients undergoing a second surgery for recurrent disease were randomized to receive an immune checkpoint inhibitor before and after surgery, or only after surgery.[3] In this study, patients receiving immune checkpoint inhibitors before and after the second surgery showed an improvement in overall survival. The proposed explanation for this is that these patients have a greater antigen load and are therefore more likely to have anticancer immune responses.

The strength of adaptive anti-cancer immune responses is a function of the immunogenicity of the cancer, the efficiency of antigen presentation, and the ability of immune cells to respond to presented antigen. Relative to other cancers, glioblastoma has a low median mutational load of 2.7 mutations per megabase of DNA,[4] suggesting low immunogenicity. However, as described in more detail below, glioblastomas elicit profound local and systemic immunosuppression: it seems very unlikely that this would occur if this cancer were not immunogenic. Antigens that are not the consequence of mutations, such as oncofetal antigens, may also contribute to the immunogenicity of this cancer. With respect to antigen presentation, glioblastoma has unique features as a consequence of its location in the brain.[5] In contrast to organs such as skin, normal brain parenchyma is not abundant in dendritic cells.[6] Adaptive immune monitoring of the brain is primarily accomplished via the meninges, which are enriched in dendritic cells and other immune cell types.[7] Cerebral interstitial fluid is filtered through the meninges; dendritic cells can take up foreign antigens there and transport them to cervical lymph nodes to present to T cells. Microglia are the predominant immune cell type in the brain parenchyma. While they are able to present antigens, they have a much reduced capacity for this compared to dendritic cells due to multiple factors including an increased capacity to degrade phagocytosed material, a reduced capacity to transport antigens out of the phagosome for presentation, and an inability to travel to draining lymph nodes (reviewed in[8]). Thus immunosurveillance, while not absent in the brain, may be less efficient than in other tissues.

Established glioblastomas repress immune cell responses by multiple mechanisms.[5] Systemically, glioblastoma patients often have very low levels of circulating T cells due to their sequestration in the bone marrow.[9] This is observed in glioblastoma and also with brain metastases, suggesting a common mechanism used by the brain to protect itself from possible adverse effects of immune cell infiltration. In spite of this, T cells are present in newly diagnosed glioblastoma tumors, comprising about 6% of the total cell population.[10] Some of these are T_{regs} with immunosuppressive functions, while others are CD4 + and CD8 + T cells in various states of energy, exhaustion, and senescence.[11] Multiple factors drive inactivation of potentially cytotoxic T cells in the glioblastoma microenvironment, including production of cytokines such as TGFβ by glioblastoma cells, production of indolamine 2,3-dioxygenase,[12,13] competition for glucose,[14] and the recruitment of T_{regs} and immunosuppressive myeloid cells. These findings derive mainly from studies on *IDH* wild-type glioblastoma, the most common form of this disease and the focus of this review. *IDH* mutant gliomas exhibit distinct mechanisms for immunosuppression. They have lower infiltration of immune cells, including microglia and macrophages, as a result of decreased cytokine expression relative to *IDH* wild-type gliomas.[15] In addition, the oncometabolite 2-hydroxyglutarate produced by mutant IDH represses T cell activity[16,17] and antigen presentation by macrophages[18] and dendritic cells.[19]

Myeloid cells are far and away the most abundant immune cell lineage in glioblastoma tumors, sometimes comprising more than half of the total cells in tumor tissue. This observation is almost a century old,[20] but recent single cell RNAseq studies have generated a much more detailed picture of this cell population.[10,21–24] Single cell RNAseq studies in glioblastoma have been reviewed comprehensively elsewhere.[25] Here I use a detailed study of IDH wild-type glioblastoma by Abdelfattah et al.[10] to illustrate the immune cell type composition of glioblastoma tumors. This group used subclustering of glioblastoma tumor immune cells to characterize myeloid cell populations.[10] They classified eight subpopulations including four microglia clusters, two macrophage clusters, a myeloid-derived suppressor cell cluster, and a small cluster of dendritic cells. A recent study has shown that dendritic cells, like cytotoxic T cells, are dysfunctional in the tumor microenvironment.[19] The two macrophage subpopulations identified by Abdelfattah et al. were both characterized as immunosuppressive, although the expression of inflammatory markers was observed in both clusters as well. Subsets of microglia also expressed both immunosuppressive and inflammatory markers. This is consistent with earlier results using bulk expression analysis[26] and reinforces the view that M1 and M2-like designations for microglia and macrophages are not applicable in glioblastoma.[27] Finally, a ninth cluster of proliferating myeloid cells was also identified, showing that some of the myeloid cells in the glioblastoma tumor microenvironment are capable of expanding their population. This study provides a detailed description of the glioblastoma tumor immune environment, with the inactivation of some immune cell types (dendritic cells, cytotoxic T cells) and active engagement of immunosuppressive immune cell types (microglia, macrophages, myeloid-derived suppressor cells, and regulatory T cells) combining to shield the tumor from the immune system. Microglia and macrophages are thought to be the principal mediators of resistance to both immune checkpoint inhibition[28] and CAR-T cell therapy[29] in glioblastoma, and understanding the mechanisms by which these cells acquire their immunosuppressive phenotype is important. The following sections discuss two general mechanisms for the generation of immunosuppressive myeloid cells in the tumor microenvironment, one involving direct communication between viable glioblastoma cells and myeloid cells, and one involving the effects of apoptotic glioblastoma cells on myeloid cells (Figure 1).

Communication Between Viable Glioblastoma Cells and Myeloid Cells

Glioblastoma cells produce chemokines that are capable of recruiting microglia and bone marrow-derived myeloid cells. Microglia and macrophages express different chemokine receptors: the former express high levels of CX3CR1 (receptor for the chemokine CX3CL1), while the latter express high levels of CCR2 (receptor for the chemokine CCL2). The highest CCL2 expression occurs in the mesenchymal molecular subtype of glioblastoma and likely explains the higher recruitment of macrophages in this subtype.[26,30] In addition to recruiting microglia and macrophages, glioblastoma cells also express cytokines that alter their immune properties. Early experiments

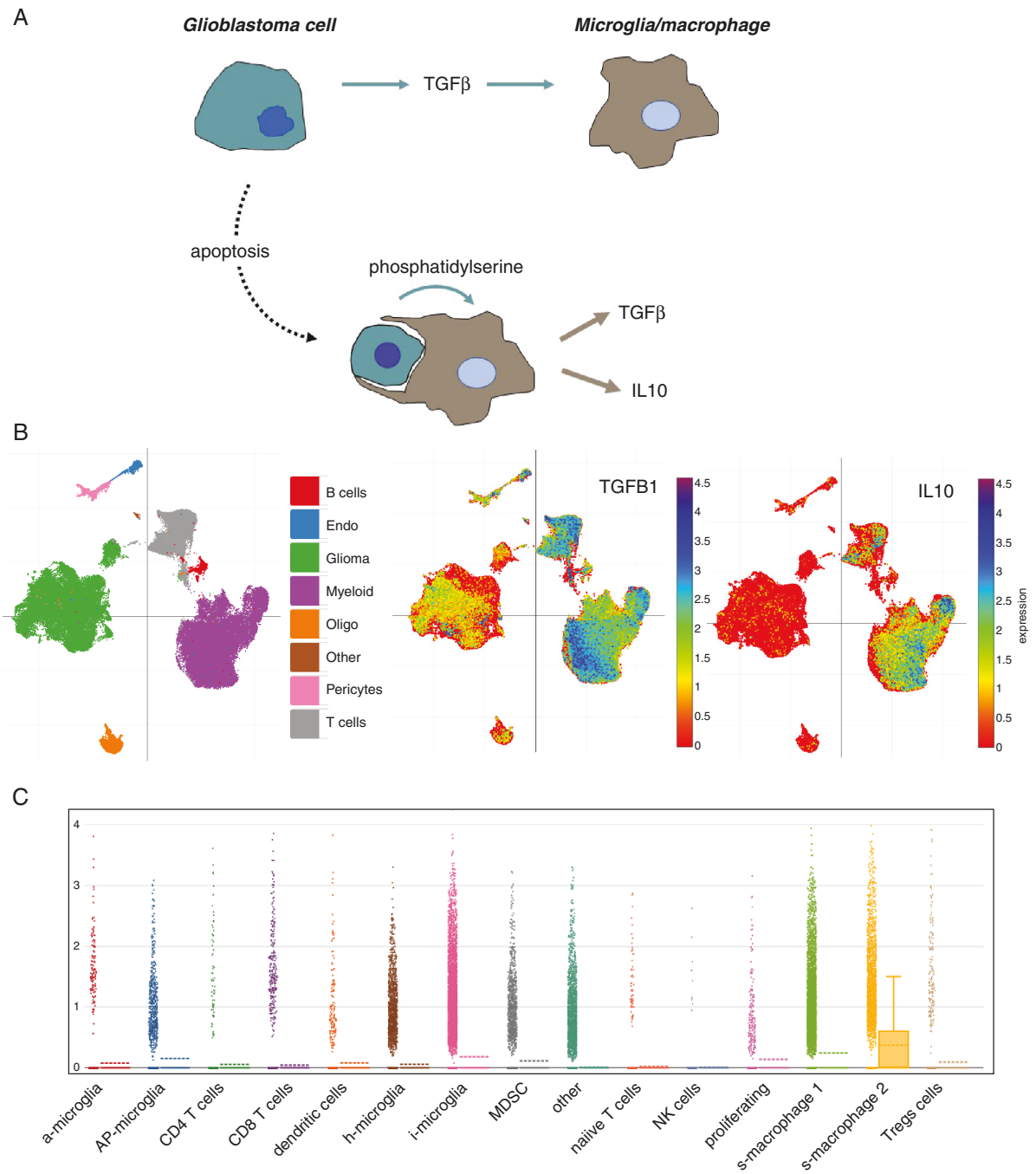

Figure 1. Mechanisms for induction of a microglia/macrophage immunosuppressive phenotype in glioblastoma tumors. (A) Viable glioblastoma cells produce factors such as transforming growth factor β(TGFβ) that induce an immunosuppressive phenotype in neighboring microglia and macrophages. Apoptotic glioblastoma cells can also potentially induce a TGFβ and IL10-secreting immunosuppressive phenotype in neighboring microglia and macrophages. Potential sources of apoptotic glioblastoma cells include necrotic regions, basal levels of glioblastoma cell apoptosis, and irradiation/temozolomide-induced apoptosis. (B) Production of immunosuppressive cytokines in glioblastoma tumor cell types. The right hand UMAP projection shows cell type assignments. The middle UMAP projection shows an expression of TGFB1in glioblastoma tumor cell types, showing that glioblastoma cells are a significant source, although myeloid cells and T cells are a larger source. Data are pooled from 18 patients, with substantial patient-to-patient variation in TGFB1 expression. The right UMAP projection shows IL10 expression. IL10 shows more restricted expression, primarily being produced by a subset of macrophages. (C) Box plots showing expression of IL10 by immune cell subtypes. The macrophage subcluster designated s-mac-2[9] shows the highest IL10 mRNA expression. Single cell RNAseq data are from Abdelfattah et al.[9] and were analyzed using the Broad Institute Single Cell portal.

showed that co-culture of glioblastoma cells with macrophages/microglia could convert the latter to an immunosuppressive phenotype.[31] This was mediated by glioblastoma cell secretion of multiple cytokines, including TGFβ. Interactions between glioblastoma cells and macrophages occur in both directions, with macrophages promoting a mesenchymal-like cell state in glioblastoma cells, which in turn promotes a mesenchymal-like cell state in macrophages.[32] Glioblastomas may also indirectly modify microglia/macrophage function via recruitment of Tregs, which respond to CCL2 signals[33] and induce an immunosuppressive phenotype in macrophages.[34] Additionally, lactate production by glioblastoma cells can promote an immunosuppressive state in tumor-resident macrophages and microglia.[35] Viable glioblastoma cells therefore have multiple mechanisms by which they can induce an immunosuppressive phenotype in tumor-resident microglia and macrophages. In normal physiology, apoptotic cells exert a powerful role in immune homeostasis that is mediated by their interaction with macrophages. Here I evaluate current evidence that this may also contribute to glioblastoma immune evasion.

Efferocytosis

As pointed out in other reviews,[36,37] billions of cells die by apoptosis every day in the human body, yet these are largely invisible as they are rapidly and efficiently cleared by macrophages, in the process known as efferocytosis. Efferocytosis is essential to the maintenance of healthy immune function, as its impairment causes severe autoimmunity.[38,39] There are two aspects of efferocytosis that contribute to this: first, efferocytosis sequesters and degrades antigens before they can elicit undesirable immune responses; second, macrophages that engage in efferocytosis acquire an immunosuppressive phenotype that includes the secretion of cytokines and other factors that repress the immune activity of other cells in the same tissue locale. The details of efferocytosis mechanisms have been reviewed recently.[36,40] The following section gives an overview of efferocytosis to provide a foundation for understanding possible roles in glioblastoma.

Efferocytosis is generally viewed as a multi-step process of attraction ("smell"), recognition ("taste"), engulfment ("eat") and phagolysosomal degradation ("digestion") (Figure 2). For the attraction step, apoptotic cells release a soluble biochemical signature that functions as a chemoattractant for phagocytes (Figure 2A). A key mechanism for the release of this biochemical signature is the activation of pannexin 1 channels by caspase-mediated cleavage.[41] The signature consists of over a 100 different metabolites, including nucleotides (eg, ATP and UTP at low concentrations) and polyamines such as spermidine. ATP functions as a chemoattractant for macrophages, signaling through their P2Y receptors.[42] Apoptotic cells can also shed a soluble form of the CX3CL1 from their cell surface,[43] which can act as a chemoattractant for phagocytes expressing the CX3CR1 receptor. These signals, in addition to acting as chemoattractants, also signal to upregulate components of the phagocytic machinery in macrophages.[36]

After chemoattraction, the next step in efferocytosis is the definitive recognition of the apoptotic cells (Figure 2B). Several signals can be used for this purpose by macrophages,[36] but extensive evidence shows that cell surface phosphatidylserine exposure is the most important of these.[44] Phosphatidylserine exposure is a direct consequence of caspase activation during apoptosis, which cleaves key proteins involved in the maintenance of phosphatidylserine exclusively in the inner leaflet of the plasma membrane.[45] Macrophages have receptors that are capable of recognizing phosphatidylserine either directly (eg, BAI1, stabilin 2, Tim-3, and 4) or indirectly via bridging proteins. The TAM family of receptor tyrosine kinases (MERTK. TYRO3 and AXL) fall into the latter category, binding to either GAS6 or PROS1 (Protein S) that have direct phosphatidylserine binding activity.

The recognition or "taste" step is followed by engulfment (Figure 2B). This stage is promoted early on by signals activated by the metabolite signature released by apoptotic cells and later by signals activated by TAM receptor tyrosine kinases. These lead to actin remodeling that is driven by RAC1 activation (reviewed in[40]). Invagination of the plasma membrane leads to the formation of the early phagosome. This undergoes a maturation process followed by fusion with the lysosome, a process coordinated by the Rab family of proteins. The final stage of efferocytosis is phagolysosomal degradation or "digestion" (Figure 2C). Apoptotic cells are broken down into component amino acids, lipids, and nucleic acids, which are then released into the macrophage cytoplasm. Multiple studies have shown that these significantly alter macrophage signaling and metabolism. Arginine and ornithine from digested apoptotic cells promote RAC1 activation in macrophages, enhancing their ability to carry out subsequent rounds of efferocytosis.[46] Fatty acids from digested apoptotic cells promote mitochondrial respiration, leading to activation of the transcription factor Pbx-1 and enhanced macrophage IL10 transcription.[47] Nucleotides from digested apoptotic cells activate DNA-dependent protein kinase, which in turn activates mTORC to drive macrophage proliferation.[48] Methionine from digested apoptotic cells provides the substrate precursor for the DNA methylase DNMT3A, which then suppresses the expression of the ERK phosphatase DUSP4 by promoter methylation; this leads to enhanced ERK signaling that promotes prostaglandin E2 production and increased TGFβ expression.[49] Digestion products from apoptotic cells therefore reprogram macrophages via changes in metabolism, transcription factor activity, and epigenetics.

The above overview of efferocytosis is mostly derived from studies on macrophages, while in glioblastoma microglia are a major component of the myeloid cell population. Efferocytosis by microglia is less well studied, but current evidence suggests that key properties described for macrophages are conserved in these cells[50] and microglia are well known to clear apoptotic cells in normal brain physiology.[51] A more detailed understanding of possible differences between microglia and bone marrow-derived macrophages with respect to efferocytosis and immunosuppression in glioblastoma will likely be achieved with the application of the comprehensive, high resolution spatial biology platforms.

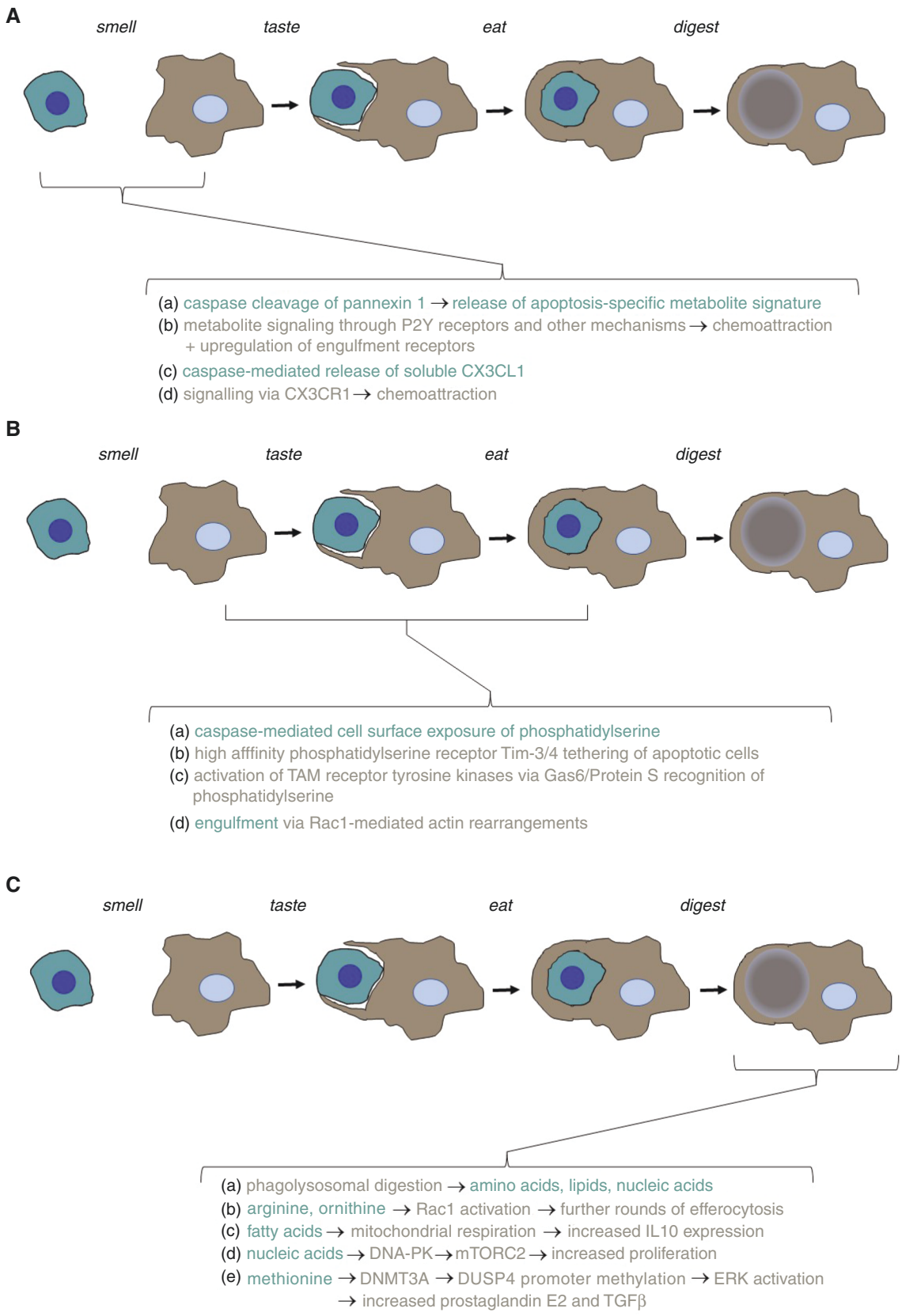

Figure 2. Signaling at different stages of efferocytosis. Schematics show different steps in efferocytosis, with apoptotic cell in teal color and efferocytosing macrophage in brown. Signaling events during the smell (A), taste/eat (B), and digest (C) steps are shown below, with contributions from the apoptotic cell in teal text and contributions from the efferocytosing macrophage shown in brown.

Efferocytosis and Immune Suppression

Alterations in signaling and metabolism during efferocytosis reprogram macrophages to an immunosuppressive state. This phenomenon was first recognized in studies on the role of neutrophils in inflammation, where phosphatidylserine exposure on the surface of apoptotic neutrophils initiates the resolution of inflammation by inducing an immunosuppressive phenotype in macrophages.[52–54] Specifically, apoptotic neutrophils promoted the production of TGFβ and IL10 by macrophages, while suppressing the expression of inflammatory cytokines. TGFβ and IL10 have broadly immunosuppressive activities; in particular, they are potent repressors of cytotoxic T cell activity, a key cell type in anti-cancer immune responses.[55,56] Further studies have identified mechanisms for the induction of an anti-inflammatory/immunosuppressive phenotype in macrophages at all stages of efferocytosis (reviewed in[57]). For example, the low concentrations of ATP released by apoptotic cells. as well as acting as a "smell" signal, can also be converted to adenosine that promotes an immunosuppressive phenotype in macrophages (reviewed in[57]); MERTK signaling during the engulfment stage inhibits pro-inflammatory Toll-like receptor signaling[58]; during the digestion phase, nuclear receptor family members, which sense changes in intracellular metabolism, also suppress the expression of immunosuppressive IL12.[59] IL10 expression is enhanced both by MERTK signaling and fatty acids released upon digestion of apoptotic cells.[47] As mentioned earlier, methionine from digested apoptotic cells promotes epigenetic changes that enhance TGFβ expression.[49]

An important aspect of this process is the amplification of immunosuppression that can occur (Figure 3). A single efferocytic cell can induce an immunosuppressive phenotype in neighboring macrophages via TGFβ and IL10 secretion; in addition, as described above, efferocytosis promotes macrophage proliferation, potentially generating an expanded pool of immunosuppressive cells. (As mentioned earlier, single cell RNAseq has identified a population of proliferating myeloid cells in glioblastoma tumors[10]; whether this arises as a result of efferocytosis or another mechanism is currently unknown.) Efferocytosis is, therefore, a potent mechanism for local immunosuppression that could be co-opted by tumors evolving to evade the immune system. Similar to this, Birge et al. have described phosphatidylserine, the key signal driving efferocytosis, as a global immunosuppressive signal in both infectious diseases and cancer.[44]

Efferocytosis as a Cancer Immune Suppression Mechanism

As in normal tissue, the extent of apoptosis in glioblastoma and other solid tumors may be masked by the rapid clearance of apoptotic cells by tumor-resident microglia and/or macrophages. Experiments in which efferocytosis is selectively inhibited in vivo can provide answers to this. Zhou et al.[60] developed an inhibitory antibody to MERTK (in a format with no effector functions) and tested this by intraperitoneal injection into a syngeneic mouse model in which MC38 murine adenocarcinoma tumors were growing subcutaneously. In this model, MERTK is only expressed on tumor-associated macrophages, not on the cancer cells. Inhibition of MERTK resulted in a significant increase in apoptotic cells. In keeping with the concept that inhibition of efferocytosis allows apoptotic cells

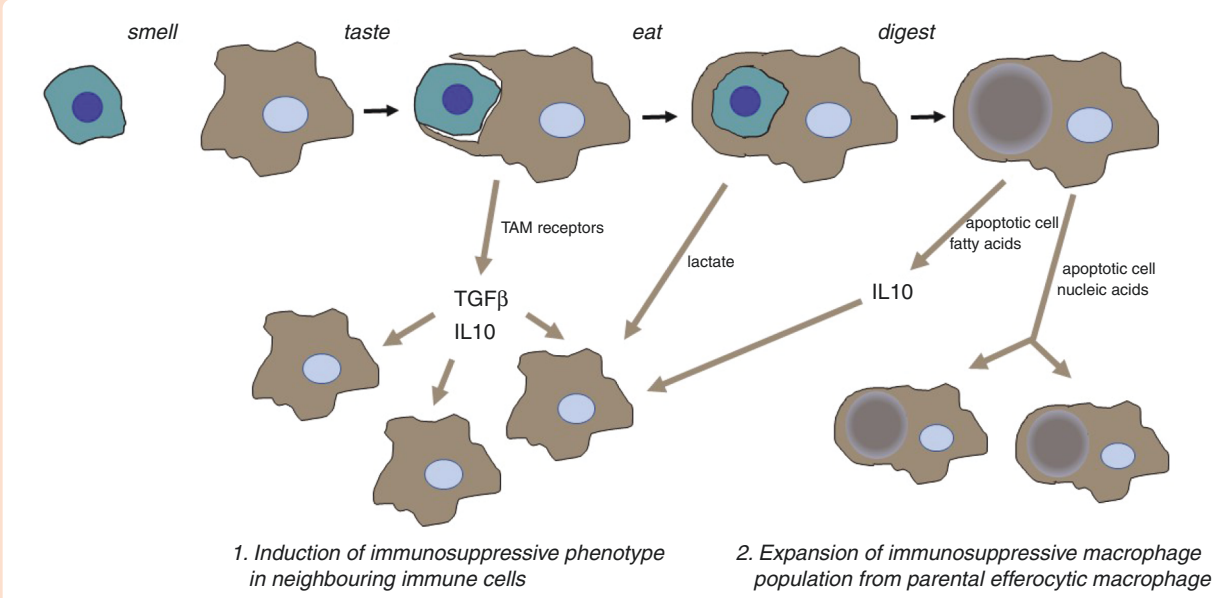

Figure 3. Immunosuppressive signal amplification with efferocytosis. Both cytokine secretion (1) and proliferation events (2) amplify the immunosuppressive signal from a single efferocytosing macrophage.

to proceed to immunogenic secondary necrosis, MERTK blockade enhanced responses to immune checkpoint inhibition. Consistent with a role for cancer cell apoptosis in this, MC38 cancer cells with mutations rendering them resistant to apoptosis were less responsive, while induction of cancer cell apoptosis with cytotoxic chemotherapy enhanced responses. In an analysis of total tumor RNA, they did not observe significant changes in either IL10 or TGFβ 3 days after antibody treatment. Necrotic cells released cGAMP which was taken up by macrophages where it activated STING. STING activation drives type I interferon expression, which in turn promotes dendritic cell (and possibly macrophage) antigen cross-presentation.[61] This study provides clear evidence for an immunosuppressive function for efferocytosis in the tumor microenvironment, albeit in a mouse model with a relatively high mutation burden.[62] The primary role of efferocytosis here appears to be preventing macrophage exposure to necrotic cancer cells releasing cGAMP. The lack of any changes in TGFβ or IL10 expression might suggest that efferocytosis is not contributing to the overall pool of these cytokines in the tumor microenvironment; however only a single time point, relatively soon after the start of MERTK inhibition, was assessed.

Efferocytosis as an Immune Suppression Mechanism in Glioblastoma

Figure 4 shows an analysis of the expression of mRNA for proteins known or proposed to have a role in the recognition of apoptotic cells during efferocytosis, making use of the glioblastoma tumor single cell RNAseq data generated by Abdelfattah et al.[10] MERTK and AXL are highly expressed at the RNA level in glioblastoma-associated microglia and macrophages. The high expression of AXL is of interest here: in the lung, it is expressed in the airway,

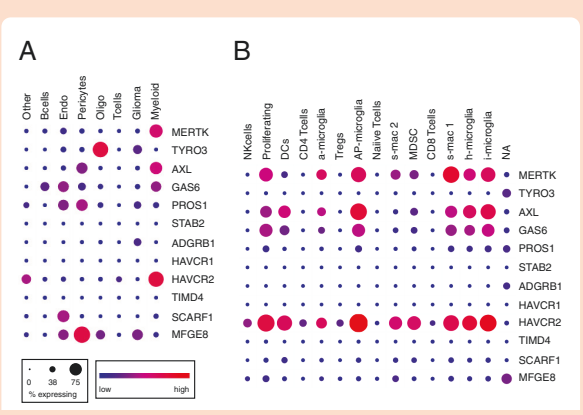

Figure 4. Phosphatidylserine receptor expression in glioblastoma. (A) Expression of phosphatidylserine binding proteins in glioblastoma tumor cell types. (B) Expression of phosphatidylserine binding proteins in glioblastoma tumor immune cell subtypes. Single cell RNAseq data are from Abdelfattah et al.[9] and were analyzed using the Broad Institute Single Cell portal.

but not interstitial macrophages, is induced by GM-CSF, type I interferon and Toll-like receptor 3 stimulation, and its absence leads to increased inflammation during influenza infection.[63] Therefore in the lung it appears to be upregulated in order to remove apoptotic cells during the resolution of inflammation. GAS6 is highly expressed, providing a link between MERTK/AXL and phosphatidylserine. Tim-3 (the product of the *HAVCR2* gene) is also highly expressed. Tim-3 binds phosphatidylserine directly and promotes efferocytosis in cell culture and in vivo.[64] It does not have a cytoplasmic tyrosine kinase domain. The cytoplasmic domain of the closely related Tim-4 protein is not required for it to promote efferocytosis,[65] indicating that it (and possibly Tim-3 as well) is involved in tethering of apoptotic cells only, and is dependent on TAM receptors for engulfment and intracellular signaling. Overall this analysis suggests that inhibition of MERTK and AXL would give effective efferocytosis inhibition in glioblastoma tumors.

Several studies have looked at the effects of AXL and MERTK inhibition on the tumor microenvironment in mouse models of glioblastoma.[66–68] Sadahiro et al.[66]treated immunocompromised mice bearing intracranial human glioblastoma xenografts with the Axl selective drug BGB324; they observed an increased number of apoptotic cells in treated tumor samples, although this was interpreted as being due to increased apoptosis rather than reduced clearance of apoptotic cells, as would be expected with efferocytosis inhibition. Wu et al.[67] tested the MerTK selective inhibitor UNC2025 in a syngeneic orthotopic mouse model that uses glioblastoma cells derived by tumor suppressor deletion and oncogene activation in murine astrocytes.[69] UNC2025 was tested alone or in combination with radiation therapy. While median survival was not affected significantly by UNC2025 either alone or in combination with radiation, a subset of mice treated with the combination treatment were long term survivors without apparent residual disease. There was a significant decrease in the percentage of CD206 + macrophages in the combination treatment group, suggesting that a shift in the macrophage population away from an immunosuppressive phenotype might explain the apparent benefit. Although efferocytosis was not addressed directly in this study and some of the effects may be due to inhibition of glioblastoma cell MERTK, overall it is very encouraging with respect to the potential of efferocytosis inhibition to improve outcomes in glioblastoma. The same group has published a second study with MRX-2843, a MERTK and FLT3 inhibitor that is undergoing clinical trials in leukemia and solid tumors (https://www.cancer.gov/about-cancer/treatment/clinical-trials/intervention/flt3-mertk-inhibitor-mrx-2843?redirect=true). In vivo evaluation was done using the GL261 syngeneic model. A decrease in CD206 + macrophage/microglia with treatment was also observed with this drug in this model.

The above studies are generally consistent with efferocytosis-mediated immunosuppression in glioblastoma, but are limited in that they make use of mouse models. Wu et al. isolated cells from glioblastoma patients and identified a subset of cells that were positive for both macrophage and glioblastoma cell signatures.[70] This was quite prevalent, in that 35% of tumor-associated macrophages showed a significant glioblastoma cell signature. The double-positive macrophages expressed

an immunosuppressive signature and were likely bone marrow-derived. Similar immunosuppressive, double-positive cells could be generated in cell culture by co-incubation of macrophages and glioblastoma cells and were the result of phagocytosis. This is relatively direct evidence for macrophage phagocytosis contributing to the glioblastoma immunosuppressive environment in human tumors. However, although the immunosuppressive macrophage phenotype is consistent with efferocytosis, the specific type of phagocytosis was not characterized in this study.

Potential Sources of Apoptotic Cells for Efferocytosis-Mediated Immunosuppression in Glioblastoma

Cancer cells are well known to develop resistance to apoptosis. This, along with the low level of detectable apoptotic cells in cancers, tends to lead to the belief that cancer cell apoptosis is negligible. However, as pointed out earlier, apoptosis is largely invisible even in normal tissues, and apoptosis resistance in cancer cells is generally partial, not absolute. Possible sources of apoptotic glioblastoma cells, both endogenous and treatment-induced, are discussed in the following sections.

Necrosis

Necrotic regions are a diagnostic feature of glioblastoma, distinguishing them from lower grade gliomas.[71,72] These regions arise as a consequence of thrombotic events in the tumor vasculature. Previously these regions were thought to be a consequence of a rapidly growing tumor outgrowing its blood supply; this view has been revised so that they are now regarded more as drivers of disease progression.[72] The presence of apoptotic cells in these regions has been shown by the detection of condensed apoptotic nuclei with hematoxylin, TUNEL labeling,[73] and cleaved caspase 3 immunohistochemistry.[74] Several studies have shown up-regulation of proteins involved in efferocytosis in macrophages situated in perinecrotic regions, including MERTK[67] and TGM2,[75] strongly suggesting that these macrophages are engaged in efferocytosis. The generation of necrotic regions is driven, at least in part, by glioblastoma cells, which produce procoagulation proteins such as tissue factor.[76] The generation of these necrotic regions is potentially a mechanism for advanced, aggressive glioblastoma tumors to activate efferocytosis and its associated immunosuppressive activity. Given that necrotic regions arise from tumor vasculature, they are more likely to be surrounded by macrophages than microglia, as perivascular macrophages are common in glioblastoma.[77] The presence of a subset of macrophages expressing high levels of IL10 in glioblastoma (Figure 1C) may be indicative of efferocytosis by perinecrotic region macrophages. Sampling for single cell RNAseq and other -omics studies typically avoids necrotic regions, so the effects of efferocytosis in necrotic regions may be underestimated in these datasets.

Basal Levels of Glioblastoma Cell Apoptosis

The study described earlier on MERTK inhibition using the MC38 murine colon tumor model showed that 1 day of in vivo inhibition of MERTK led to a substantial increase in apoptotic cells within the tumor.[60] This points to high levels of intrinsic apoptosis and efferocytic clearance in this murine model. Whether this also occurs in glioblastoma (outside of necrotic areas) is not known. Given the well-documented heterogeneity of glioblastoma, this may vary substantially from patient-to-patient and within patient tumors. As different cancer driver mutations are able to repress apoptosis to different degrees, it may be that certain glioblastoma mutational signatures are associated with lower or higher levels of basal apoptosis and might therefore be predictive of benefit from efferocytosis inhibition. The basic principle for this was nicely demonstrated by Zhou et al., who showed that MC38 cells with deletions in key pro-apoptotic genes were no longer responsive to MERTK inhibition in vivo.[60]

Radiation and Temozolomide

The combination of radiation and temozolomide is standard-of-care in glioblastoma treatment.[1] Radiation combined with immunotherapy has been proposed as a possible effective combination for cancer treatment in general[78] and in glioblastoma.[79] However there is evidence for radiation having both immunostimulatory and immunosuppressive effects.[78] Efferocytic clearance of apoptotic cells post radiation may be a key mechanism that limits the immunostimulatory effects of radiation. Crittenden et al. investigated the effects of MERTK knockout on radiation responses.[80] In the immunogenic CT26 murine colon cancer model, radiation treatment produced long term survivors in MERTK knockout mice, but not in control mice. This effect was much weaker in the poorly immunogenic Panc02 model but was markedly enhanced with the addition of a TGFβ receptor inhibitor. Toerman et al. also showed improved survival in the CT26 model with MERTK knockout and radiation and that this effect was dependent on the presence of CD8 T cells.[81] As discussed earlier, Wu et al. assessed the effects of combining MERTK inhibition with radiation in an orthotopic syngeneic mouse model of glioblastoma,[67] showing that a subset of mice in the combined treatment arm survived longer than 60 days, most with no evidence of disease, while there were no survivors in control or single treatment arms at this time point. These studies suggest that efferocytosis and its immunosuppressive activity inhibit immune responses in radiation therapy, both in standard syngeneic mouse models and in a more clinically relevant glioblastoma model.

Temozolomide induces both apoptosis and senescence in glioblastoma cells in cell culture with some evidence pointing to this occurring in vivo as well (reviewed in[82]), suggesting that this could also be a source of apoptotic cells to initiate efferocytosis. Targeted therapies, particularly EGF receptor tyrosine kinase inhibitors, have been unsuccessful in glioblastoma.[83] This is attributed to redundancy in the activation of key signaling pathways, with multiple alternate tyrosine kinase receptors and PTEN loss being able

to substitute for EGF receptor activity. EGFR receptor tyrosine kinases inhibitors are able to induce apoptosis of some glioblastoma cells.[84] This area could potentially be revisited in combination with efferocytosis inhibition, where the goal would be to induce sufficient immunogenic glioblastoma cell death to induce an immune response.

Pharmacology of Efferocytosis Inhibition in Glioblastoma

Overall strategies to target efferocytosis have been comprehensively reviewed recently[36]; this section addresses studies specific to glioblastoma. The TAM kinases MERTK, TYRO3, and AXL have a central role in efferocytosis and their tyrosine kinase activity makes them readily druggable targets. As mentioned earlier, the MERTK inhibitor UNC2025 was tested in a preclinical orthotopic glioblastoma model. An interesting observation from this study was the high levels of UNC2025 that were achieved in brain tumor tissue. As discussed by the authors, this may be due to the properties of the drug, but might also be influenced by the secondary role of MERTK in maintaining the blood-brain barrier,[85] which would be an instance of pharmacologic serendipity for efferocytosis inhibition. In addition to inhibiting MERTK, UNC2025, and MRX-2843 have potent activity against Flt3; possible effects due to inhibition of this target in glioblastoma are unknown. These compounds have lower activity against AXL, which the single cell RNAseq analysis in Figure 4 suggests may share a role with MERTK in glioblastoma-associated macrophage efferocytosis. A comparison with other MerTK inhibitors, such as ONO-7475 which has high potency for MERTK and Axl and low potency for FLT3, might be informative. Several of these drugs have been evaluated or are under evaluation in clinical trials for other cancers, primarily myelogenous leukemia, but also lung cancer. Other strategies to inhibit TAM receptors are also being explored, including inhibitory antibodies to MERTK[60] and an engineered version of the AXL receptor extracellular domain that acts as a GAS6 decoy.[86] These might apply to glioblastoma if effective delivery can be achieved.

An alternate approach to efferocytosis inhibition may be the use of antibodies to phosphatidylserine. The mouse/human chimeric antibody bavituximab was originally developed to selectively target tumor vasculature.[87] It targets cell surface phosphatidylserine complexed with β2 glycoprotein I which is selectively present in tumor vasculature. In the F98 cell rat model, irradiation increased phosphatidylserine expression on both tumor vasculature endothelial cells and F98 glioma cells; however, this was attributed to oxidative stress effects rather than apoptosis.[88] In the same model, the combination of bavituximab and irradiation improved survival. A subset of rats were long term survivors and were resistant to rechallenge with F98 cells. While bavituximab partly acts by targeting tumor vasculature destruction, the induction of long term immunity is consistent with the repression of efferocytosis-like signaling events in macrophages. A recent study evaluated bavituximab in 36 newly diagnosed glioblastoma patients.[89] They observed a significant decrease in myeloid-derived suppressor cell numbers in tumors after bavituximab treatment, a finding that had previously been observed in animal models.[87] This suggests that some interference with phosphatidylserine signaling to myeloid cells was achieved in human glioblastoma. Low levels of antibody penetration into the brain and tumor may limit the ability of bavituximab to act by this mechanism without undesirable side effects.

Outstanding Questions

As discussed in the Introductory section, the small clinical trial of neoadjuvant PD-1 inhibition suggests that glioblastoma may be sensitive to immunotherapy if there is sufficient antigen exposure. There is solid evidence for the presence of apoptotic cells in glioblastoma necrotic regions and also with radiation treatment. Preventing efferocytosis so that these cells progress to secondary necrosis may be an effective way to increase antigen exposure. As with PD-1 inhibition, efferocytosis inhibition would be predicted to be far more effective if given prior to surgery. A better understanding of basal levels of apoptosis in glioblastoma tumors might also be helpful in knowing how best to test efferocytosis inhibition strategies in patients. The development of methodologies for identifying efferocytosis in single cell RNA sequencing data, perhaps based on the double-positive approach used by Wu et al.,[70] would be very helpful in this regard. For cancer therapy, effective efferocytosis inhibition would induce anti-tumor immunity while avoiding toxicity due to autoimmunity. Whether selective MERTK inhibition, broader inhibition of TAM receptors, or phosphatidylserine blockade is the best approach to achieving this is an important question. It is also possible that the two pathways indicated in Figure 1 operate redundantly and that efferocytosis inhibition would need to be combined with TGFβ signal inhibition to induce immune responses, as observed by Crittenden et al. in a murine pancreatic cancer model.[80] Further preclinical studies in clinically relevant glioblastoma models may help address these issues.

Keywords

efferocytosis | glioblastoma | glioma | immune | macrophage | phagocytosis

Funding

Canadian Institutes of Health Research (162180).

Conflict of interest

The author declares no conflict of interest.

Acknowledgments

Thanks to Dr. Michele Ardolino, Ottawa Hospital Research Institute, and the University of Ottawa, for comments on the manuscript.

Authorship statement

This review was conceived and written by the author.

List of unpublished papers cited

1. Gupta P, Dang M, Oberai S, et al. Immune landscape of isocitrate dehydrogenase stratified human gliomas *BioRxiv.* 2022;11.08.514794.
2. Miller TE, Farran CAE, Couturier CP, et al. Programs, Origins, and Niches of Immunomodulatory Myeloid Cells in Gliomas *BioRxiv.* 2023;10.24.563466.

References

1. Stupp R, Mason WP, van den Bent MJ, et al.; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352(10):987–996.
2. Reardon DA, Brandes AA, Omuro A, et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: The CheckMate 143 phase 3 randomized clinical trial. *JAMA Oncol.* 2020;6(7):1003–1008.
3. Cloughesy TF, Mochizuki AY, Orpilla JR, et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. *Nat Med.* 2019;25(3):477–486.
4. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med.* 2017;9(1):34.
5. Sampson JH, Gunn MD, Fecci PE, Ashley DM. Brain immunology and immunotherapy in brain tumours. *Nat Rev Cancer.* 2020;20(1):12–25.
6. D'Agostino PM, Gottfried-Blackmore A, Anandasabapathy N, Bulloch K. Brain dendritic cells: biology and pathology. *Acta Neuropathol.* 2012;124(5):599–614.
7. Alves de Lima K, Rustenhoven J, Kipnis J. Meningeal immunity and its function in maintenance of the central nervous system in health and disease. *Annu Rev Immunol.* 2020;38:597–620.
8. Lecoultre M, Dutoit V, Walker PR. Phagocytic function of tumor-associated macrophages as a key determinant of tumor progression control: a review. *J ImmunoTher Cancer.* 2020;8(2):e001408.
9. Chongsathidkiet P, Jackson C, Koyama S, et al. Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors. *Nat Med.* 2018;24(9):1459–1468.
10. Abdelfattah N, Kumar P, Wang C, et al. Single-cell analysis of human glioma and immune cells identifies S100A4 as an immunotherapy target. *Nat Commun.* 2022;13(1):767.
11. Woroniecka KI, Rhodin KE, Chongsathidkiet P, Keith KA, Fecci PE. T-cell dysfunction in glioblastoma: applying a new framework. *Clin Cancer Res.* 2018;24(16):3792–3802.
12. Wainwright DA, Balyasnikova IV, Chang AL, et al. IDO expression in brain tumors increases the recruitment of regulatory T cells and negatively impacts survival. *Clin Cancer Res.* 2012;18(22):6110–6121.
13. Uyttenhove C, Pilotte L, Théate I, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nat Med.* 2003;9(10):1269–1274.
14. Mirzaei R, Sarkar S, Yong VW. T cell exhaustion in glioblastoma: intricacies of immune checkpoints. *Trends Immunol.* 2017;38(2):104–115.
15. Amankulor NM, Kim Y, Arora S, et al. Mutant IDH1 regulates the tumor-associated immune system in gliomas. *Genes Dev.* 2017;31(8):774–786.
16. Bunse L, Pusch S, Bunse T, et al. Suppression of antitumor T cell immunity by the oncometabolite (R)-2-hydroxyglutarate. *Nat Med.* 2018;24(8):1192–1203.
17. Notarangelo G, Spinelli JB, Perez EM, et al. Oncometabolite d-2HG alters T cell metabolism to impair CD8(+) T cell function. *Science.* 2022;377(6614):1519–1529.
18. Friedrich M, Sankowski R, Bunse L, et al. Tryptophan metabolism drives dynamic immunosuppressive myeloid states in IDH-mutant gliomas. *Nat Cancer.* 2021;2(7):723–740.
19. Friedrich M, Hahn M, Michel J, et al. Dysfunctional dendritic cells limit antigen-specific T cell response in glioma. *Neuro Oncol.* 2023;25(2):263–276.
20. Penfield W. Microglia and the process of phagocytosis in gliomas. *Am J Pathol.* 1925;1(1):77–90.15.15.
21. Pombo Antunes AR, Scheyltjens I, Lodi F, et al. Single-cell profiling of myeloid cells in glioblastoma across species and disease stage reveals macrophage competition and specialization. *Nat Neurosci.* 2021;24(4):595–610.
22. Blanco-Carmona E, Narayanan A, Hernandez I, et al. Tumor heterogeneity and tumor-microglia interactions in primary and recurrent IDH1-mutant gliomas. *Cell Rep Med.* 2023;4(11):101249.
23. Gupta P, Dang M, Oberai S, et al. Immune landscape of isocitrate dehydrogenase stratified human gliomas. *BioRxiv.* 2022;11.08.514794.
24. Miller TE, Farran CAE, Couturier CP, et al. Programs, origins, and niches of immunomodulatory myeloid cells in gliomas. *BioRxiv.* 2023;10.24.563466.
25. Eisenbarth D, Wang YA. Glioblastoma heterogeneity at single cell resolution. *Oncogene.* 2023;42(27):2155–2165.
26. Gabrusiewicz K, Rodriguez B, Wei J, et al. Glioblastoma-infiltrated innate immune cells resemble M0 macrophage phenotype. *JCI Insight.* 2016;1(2):e85841.
27. Wei J, Chen P, Gupta P, et al. Immune biology of glioma-associated macrophages and microglia: functional and therapeutic implications. *Neuro Oncol.* 2020;22(2):180–194.
28. Lee AH, Sun L, Mochizuki AY, et al. Neoadjuvant PD-1 blockade induces T cell and cDC1 activation but fails to overcome the immunosuppressive tumor associated macrophages in recurrent glioblastoma. *Nat Commun.* 2021;12(1):6938.
29. Yang F, Zhang D, Jiang H, et al. Small-molecule toosendanin reverses macrophage-mediated immunosuppression to overcome glioblastoma resistance to immunotherapy. *Sci Transl Med.* 2023;15(683):eabq3558.
30. Engler JR, Robinson AE, Smirnov I, et al. Increased microglia/macrophage gene expression in a subset of adult and pediatric astrocytomas. *PLoS One.* 2012;7(8):e43339.
31. Wu A, Wei J, Kong LY, et al. Glioma cancer stem cells induce immunosuppressive macrophages/microglia. *Neuro Oncol.* 2010;12(11):1113–1125.
32. Hara T, Chanoch-Myers R, Mathewson ND, et al. Interactions between cancer cells and immune cells drive transitions to mesenchymal-like states in glioblastoma. *Cancer Cell.* 2021;39(6):779–792.e11.

33. Jin J, Lin J, Xu A, et al. CCL2: an important mediator between tumor cells and host cells in tumor microenvironment. *Front Oncol.* 2021;11:722916.
34. Tiemessen MM, Jagger AL, Evans HG, et al. CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. *Proc Natl Acad Sci U S A.* 2007;104(49):19446–19451.
35. Goswami KK, Banerjee S, Bose A, Baral R. Lactic acid in alternative polarization and function of macrophages in tumor microenvironment. *Hum Immunol.* 2022;83(5):409–417.
36. Mehrotra P, Ravichandran KS. Drugging the efferocytosis process: Concepts and opportunities. *Nat Rev Drug Discov.* 2022;21(8):601–620.
37. Arandjelovic S, Ravichandran KS. Phagocytosis of apoptotic cells in homeostasis. *Nat Immunol.* 2015;16(9):907–917.
38. Asano K, Miwa M, Miwa K, et al. Masking of phosphatidylserine inhibits apoptotic cell engulfment and induces autoantibody production in mice. *J Exp Med.* 2004;200(4):459–467.
39. Lu Q, Lemke G. Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family. *Science.* 2001;293(5528):306–311.
40. Boada-Romero E, Martinez J, Heckmann BL, Green DR. The clearance of dead cells by efferocytosis. *Nat Rev Mol Cell Biol.* 2020;21(7):398–414.
41. Medina CB, Mehrotra P, Arandjelovic S, et al. Metabolites released from apoptotic cells act as tissue messengers. *Nature.* 2020;580(7801):130–135.
42. Elliott MR, Chekeni FB, Trampont PC, et al. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature.* 2009;461(7261):282–286.
43. Truman LA, Ford CA, Pasikowska M, et al. CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. *Blood.* 2008;112(13):5026–5036.
44. Birge RB, Boeltz S, Kumar S, et al. Phosphatidylserine is a global immunosuppressive signal in efferocytosis, infectious disease, and cancer. *Cell Death Differ.* 2016;23(6):962–978.
45. Nagata S, Suzuki J, Segawa K, Fujii T. Exposure of phosphatidylserine on the cell surface. *Cell Death Differ.* 2016;23(6):952–961.
46. Yurdagul A, Jr, Subramanian M, Wang X, et al. Macrophage metabolism of apoptotic cell-derived arginine promotes continual efferocytosis and resolution of injury. *Cell Metab.* 2020;31(3):518–533.e10.
47. Zhang S, Weinberg S, DeBerge M, et al. Efferocytosis fuels requirements of fatty acid oxidation and the electron transport chain to polarize macrophages for tissue repair. *Cell Metab.* 2019;29(2):443–456.e5.
48. Gerlach BD, Ampomah PB, Yurdagul A, Jr, et al. Efferocytosis induces macrophage proliferation to help resolve tissue injury. *Cell Metab.* 2021;33(12):2445–2463.e8.
49. Ampomah PB, Cai B, Sukka SR, et al. Macrophages use apoptotic cell-derived methionine and DNMT3A during efferocytosis to promote tissue resolution. *Nat Metab.* 2022;4(4):444–457.
50. Márquez-Ropero M, Benito E, Plaza-Zabala A, Sierra A. Microglial corpse clearance: lessons from macrophages. *Front Immunol.* 2020;11:506.
51. Zhao J, Zhang W, Wu T, et al. Efferocytosis in the central nervous system. *Front Cell Dev Biol.* 2021;9:773344.
52. Voll RE, Herrmann M, Roth EA, et al. Immunosuppressive effects of apoptotic cells. *Nature.* 1997;390(6658):350–351.
53. Filardy AA, Pires DR, Nunes MP, et al. Proinflammatory clearance of apoptotic neutrophils induces an IL-12(low)IL-10(high) regulatory phenotype in macrophages. *J Immunol (Baltimore, Md. : 1950).* 2010;185(4):2044–2050.
54. Fadok VA, Bratton DL, Konowal A, et al. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest.* 1998;101(4):890–898.
55. Thomas DA, Massagué J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell.* 2005;8(5):369–380.
56. Dennis KL, Blatner NR, Gounari F, Khazaie K. Current status of interleukin-10 and regulatory T-cells in cancer. *Curr Opin Oncol.* 2013;25(6):637–645.
57. Elliott MR, Koster KM, Murphy PS. Efferocytosis signaling in the regulation of macrophage inflammatory responses. *J Immunol (Baltimore, Md. : 1950).* 2017;198(4):1387–1394.
58. Rothlin CV, Ghosh S, Zuniga EI, Oldstone MB, Lemke G. TAM receptors are pleiotropic inhibitors of the innate immune response. *Cell.* 2007;131(6):1124–1136.
59. Ipseiz N, Uderhardt S, Scholtysek C, et al. The nuclear receptor Nr4a1 mediates anti-inflammatory effects of apoptotic cells. *J Immunol (Baltimore, Md. : 1950).* 2014;192(10):4852–4858.
60. Zhou Y, Fei M, Zhang G, et al. Blockade of the phagocytic receptor MerTK on tumor-associated macrophages enhances P2X7R-dependent STING activation by tumor-derived cGAMP. *Immunity.* 2020;52(2):357–373.e9.
61. Lorenzi S, Mattei F, Sistigu A, et al. Type I IFNs control antigen retention and survival of CD8α(+) dendritic cells after uptake of tumor apoptotic cells leading to cross-priming. *J Immunol (Baltimore, Md. : 1950).* 2011;186(9):5142–5150.
62. Zhong W, Myers JS, Wang F, et al. Comparison of the molecular and cellular phenotypes of common mouse syngeneic models with human tumors. *BMC Genomics.* 2020;21(1):2.
63. Fujimori T, Grabiec AM, Kaur M, et al. The Axl receptor tyrosine kinase is a discriminator of macrophage function in the inflamed lung. *Mucosal Immunol.* 2015;8(5):1021–1030.
64. Nakayama M, Akiba H, Takeda K, et al. Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. *Blood.* 2009;113(16):3821–3830.
65. Park D, Hochreiter-Hufford A, Ravichandran KS. The phosphatidylserine receptor TIM-4 does not mediate direct signaling. *Curr Biol.* 2009;19(4):346–351.
66. Sadahiro H, Kang KD, Gibson JT, et al. Activation of the receptor tyrosine kinase AXL regulates the immune microenvironment in glioblastoma. *Cancer Res.* 2018;78(11):3002–3013.
67. Wu J, Frady LN, Bash RE, et al. MerTK as a therapeutic target in glioblastoma. *Neuro Oncol.* 2018;20(1):92–102.
68. Su YT, Butler M, Zhang M, et al. MerTK inhibition decreases immune suppressive glioblastoma-associated macrophages and neoangiogenesis in glioblastoma microenvironment. *Neuro Oncol Adv.* 2020;2(1):vdaa065.
69. Schmid RS, Simon JM, Vitucci M, et al. Core pathway mutations induce de-differentiation of murine astrocytes into glioblastoma stem cells that are sensitive to radiation but resistant to temozolomide. *Neuro Oncol.* 2016;18(7):962–973.
70. Wu M, Wu L, Wu W, et al. Phagocytosis of glioma cells enhances the immunosuppressive phenotype of bone marrow-derived macrophages. *Cancer Res.* 2023;83(5):771–785.
71. Rong Y, Durden DL, Van Meir EG, Brat DJ. 'Pseudopalisading' necrosis in glioblastoma: a familiar morphologic feature that links vascular pathology, hypoxia, and angiogenesis. *J Neuropathol Exp Neurol.* 2006;65(6):529–539.
72. Markwell SM, Ross JL, Olson CL, Brat DJ. Necrotic reshaping of the glioma microenvironment drives disease progression. *Acta Neuropathol.* 2022;143(3):291–310.
73. Tachibana O, Lampe J, Kleihues P, Ohgaki H. Preferential expression of Fas/APO1 (CD95) and apoptotic cell death in perinecrotic cells of glioblastoma multiforme. *Acta Neuropathol.* 1996;92(5):431–434.
74. Brat DJ, Castellano-Sanchez AA, Hunter SB, et al. Pseudopalisades in glioblastoma are hypoxic, express extracellular matrix proteases, and are formed by an actively migrating cell population. *Cancer Res.* 2004;64(3):920–927.
75. Lorimer I, Elgafarawi M, Lui M, Sevinc F, et al. Transglutaminase 2 expression in glioblastoma: evidence for a role in efferocytosis. *Neuro Oncol Adv.* 2023;25(Supp5):v290.

76. Magnus N, D'Asti E, Garnier D, Meehan B, Rak J. Brain neoplasms and coagulation. *Semin Thromb Hemost*. 2013;39(8):881–895.

77. Sørensen MD, Kristensen BW. Tumour-associated CD204(+) microglia/macrophages accumulate in perivascular and perinecrotic niches and correlate with an interleukin-6-enriched inflammatory profile in glioblastoma. *Neuropathol Appl Neurobiol*. 2022;48(2):e12772.

78. Weichselbaum RR, Liang H, Deng L, Fu YX. Radiotherapy and immunotherapy: a beneficial liaison? *Nat Rev Clin Oncol*. 2017;14(6):365–379.

79. Nesseler JP, Schaue D, McBride WH, et al. Irradiation to improve the response to immunotherapeutic agents in glioblastomas. *Adv Radiat Oncol*. 2019;4(2):268–282.

80. Crittenden MR, Baird J, Friedman D, et al. Mertk on tumor macrophages is a therapeutic target to prevent tumor recurrence following radiation therapy. *Oncotarget*. 2016;7(48):78653–78666.

81. Tormoen GW, Blair TC, Bambina S, et al. Targeting MerTK enhances adaptive immune responses after radiation therapy. *Int J Radiat Oncol Biol Phys*. 2020;108(1):93–103.

82. Kaina B. Temozolomide, procarbazine and nitrosoureas in the therapy of malignant gliomas: update of mechanisms, drug resistance and therapeutic implications. *J Clin Med*. 2023;12(23):7442.

83. van den Bent MJ, Brandes AA, Rampling R, et al. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. *J Clin Oncol*. 2009;27(8):1268–1274.

84. Geoerger B, Gaspar N, Opolon P, et al. EGFR tyrosine kinase inhibition radiosensitizes and induces apoptosis in malignant glioma and childhood ependymoma xenografts. *Int J Cancer*. 2008;123(1):209–216.

85. Miner JJ, Daniels BP, Shrestha B, et al. The TAM receptor Mertk protects against neuroinvasive viral infection by maintaining blood-brain barrier integrity. *Nat Med*. 2015;21(12):1464–1472.

86. Kariolis MS, Miao YR, Jones DS, 2nd, et al. An engineered Axl 'decoy receptor' effectively silences the Gas6-Axl signaling axis. *Nat Chem Biol*. 2014;10(11):977–983.

87. Yin Y, Huang X, Lynn KD, Thorpe PE. Phosphatidylserine-targeting antibody induces M1 macrophage polarization and promotes myeloid-derived suppressor cell differentiation. *Cancer Immunol Res*. 2013;1(4):256–268.

88. He J, Yin Y, Luster TA, Watkins L, Thorpe PE. Antiphosphatidylserine antibody combined with irradiation damages tumor blood vessels and induces tumor immunity in a rat model of glioblastoma. *Clin Cancer Res*. 2009;15(22):6871–6880.

89. Ly KI, Richardson LG, Liu M, et al. Bavituximab decreases immunosuppressive myeloid-derived suppressor cells in newly diagnosed glioblastoma patients. *Clin Cancer Res*. 2023;29(16):3017–3025.