All in the head: obstacles for immune rejection of brain tumours

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INTRODUCTION

Tumour immunology and immunotherapy is a highly active field, a clinical testing ground for cutting edge immunological techniques and concepts. But this is after many years of fundamental advances in basic immunology. In this article we suggest that immunotherapy for brain tumours cannot be rationally advanced as rapidly as that for tumours in other sites. Our understanding of anti-tumour immune responses in the brain is sketchy and frequently extrapolated from other tissues having little in common with the central nervous system (CNS). The result is that current clinical trials are built upon shakier foundations, with the somewhat naive optimism that what is looking hopeful for other tumours will also be applicable to cerebral malignancies. But of course it is easy to criticise such well-meaning attempts to treat currently incurable cancers. In the basic and preclinical domain, brain tumour models that are readily applicable to the design of future immunotherapies are only in their infancy. The ideal transplantable tumour that reiterates the key features of a malignant primary astrocytoma (poorly immunogenic, infiltrative but non-metastatic, expressing multiple mechanisms mediating immune escape) has yet to be discovered. In the meantime, we must use individual model tumours and limit the scope of the conclusions that we make from each system. Moreover, we must overcome the significant technical difficulties encountered as we strive to preserve brain integrity, whilst implanting tumours in this unique site. Or we can look to genetic models, in which there is the development of 'spontaneous' brain tumours (in some cases aided by the intracerebral delivery of a viral vector) incorporating many of the genetic features and heterogeneity typical of spontaneous human cancer.[1]

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Abbreviations: APC, antigen-presenting cell; BBB, blood–brain barrier; CNS, central nervous system; CTL, cytotoxic T lymphocyte; IFN, interferon; IL, interleukin; PG, prostaglandin; TIL, tumour-infiltrating lymphocyte; TGF, transforming growth factor.

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However, these models have generally been constructed to address genetic and pathological issues and they present a significant challenge for interpretable immunological studies.

With these difficulties in mind, perhaps we need to accept that brain tumour models and clinical immunotherapies are currently in their first generation. To progress to a more successful second generation of therapies, there is a need to abandon the idea that an automatic one-way progression exists from rodent models to the clinic. We need better models to make better therapies, but how to choose and design the models can be greatly guided by data from clinical trials, if the trial design actually permits the generation of useful biological data. To date, most brain tumour immunotherapies have borrowed technologies and approaches already explored for tumours in other sites, principally melanoma. Thus, most of the now 'classical' tumour immunology approaches have been explored for brain tumour immunotherapy: cytokine immune enhancement, whole tumour cell vaccines, cytokine-modified tumour cell vaccines, gene therapies with immune bystander effects, dendritic cell therapies (reviewed in refs. 2–4). A few notable and exciting novel approaches unique to the CNS should not be passed aside, for example, exploiting the migratory properties of neural stem cells to deliver immunoactive molecules efficiently to the tumour site.[5] The overall conclusions from these studies are that, depending upon the stringency of the models utilized, tumours that are pre-established in the brain are generally more difficult to eliminate than those in other sites, and may require different effector mechanisms. For example, studies in which multiple cytokines have been tested as modulators of immune responses gave different results according to the tumour model (SMA-560, B16) and site of implantation.[6,7] Other attempts to create a cellular vaccine by overexpression of intercellular adhesion molecule-1 on a glioma cell line, resulted in growth inhibition of glioma cells implanted subcutaneously, but not in the CNS.[8] Another recent study using a recombinant *Listeria monocytogenes* tumour vaccine revealed more stringent T-cell subset requirements for protection against an intracerebral challenge compared with the same tumour implanted by the subcutaneous route.[9] These examples, together with the fact that no convincingly successful clinical brain tumour immunotherapy has been

demonstrated to date, should force us to reassess what we understand about brain tumour immunology, rather than just brain tumour immunotherapy. It is from this perspective that we will discuss the issues pertinent to the problem by drawing from both clinical and experimental situations.

THE CLINICAL PROBLEM

Both primary brain tumours and intracranial metastases pose a serious clinical problem, although their involvement with the host immune system will presumably have followed a different evolution. Concerning primary brain neoplasms, those derived from astrocytes are the most frequent, and the anaplastic astrocytomas and glioblastomas (grade III and IV astrocytomas, according to the WHO designation) are the most lethal. Indeed, despite some advances in surgical resection, radiotherapy and chemotherapy,[10] it is unlikely that long-term survival rates can be significantly extended beyond the current median survival of less than 12 months for glioblastomas.[11] Furthermore, although for low-grade astrocytomas the outlook is more favourable, not all can be adequately treated and they may progress to malignant lesions.[1] Malignant astrocytomas infiltrate normal tissue, which renders total surgical resection virtually impossible without extensive neurological damage. It is thus essential to consider novel treatments such as immunotherapy in the hope of attacking the residual radioresistant and chemoresistant tumour cells. Other tumours metastasizing to the brain, such as melanoma, pose similar problems,[12] and for these tumours it would also be useful to propose a therapeutic option that is applicable to the CNS.

THE PARTICULAR REQUIREMENTS FOR IMMUNE RESPONSES AGAINST TUMOURS IN THE CNS – A SITE OF IMMUNE PRIVILEGE

Whilst certain criteria for acceptable anti-tumour responses are applicable to all tumours, there are other requirements that are more stringent for cerebral malignancies. Indeed, the categorization of the CNS as an immune privileged site has perhaps retarded the development of immunotherapies for brain tumours, with the temptation to anticipate ineffective immune function in the brain. However, this prejudgement of the domain has arisen from a degree of misunderstanding of immune privilege. The term originally arose to describe the results of transplantation experiments in which there was extended survival of tissues transplanted to the CNS, compared with their survival in other sites.[13,14] Features of the CNS were identified (certain of which are discussed individually in the sections below) that were proposed to explain this apparent lack of immune reactivity. These included the presence of the blood–brain barrier (BBB), low major histocompatibility complex (MHC) expression in the brain parenchyma, the absence of organized lymphatic drainage and a lack of dendritic cells in the normal brain parenchyma. Nevertheless, it is now apparent that immune reactions can and do occur in the CNS: autoimmune diseases of the CNS;[15] immune responses to neurotropic viruses[16] and parasites;[17] and, as will be discussed herein, anti-tumour responses. Immune privilege is thus a term that requires an updated definition: it should remind us that although immune responses in the brain are often qualitatively and quantitatively different to those found in other sites, they are not absent. As elegantly phrased by Fabry *et al.*,[18] we have to consider immune responses in the CNS as having a certain 'dialect'.

Those aspects of immune privilege that impinge on afferent immune responses are clearly of fundamental interest, but may be less critical for brain tumour immunotherapy. For most vaccine strategies, immune responses will be induced at sites remote from the tumour, with the aim that effector cells can then recirculate to mediate their anti-tumour effects in the brain. The discussions that follow will therefore concentrate on those particularities of the CNS that influence the efferent arm of anti-tumour immune responses (Fig. 1), with a bias towards those that are presumed to be T-cell-mediated.

Effector T cells must penetrate the brain parenchyma before they can reach the tumour bed

The first requirement for an effector T cell is that it must reach its target, the tumour. The problem of adequate tumour infiltration is applicable to all solid cancers.[19,20] but when the tumour is located in the brain parenchyma, the T cell must also penetrate the tight junctions between the endothelial cells of the cerebral vasculature constituting the BBB.[21,22] The integrity of this barrier is maintained by cells in intimate contact with the abluminal surface of the endothelium. Pericytes, perivascular cells and particularly astrocytes are implicated, the latter cells almost totally surrounding the vessel with their foot processes.[23] Whilst the brain microvessels constituting the BBB appear impermeable compared with other microvessels, the barrier is conditional and selective. There is molecular and cellular traffic in both directions, but this is tightly regulated. Activated T cells can extravasate, but their trafficking may be more limited than for other sites. For example, the relative number of activated T cells found in the brain parenchyma after intravenous adoptive transfer of labelled T cells in rats was six times less than in muscle and more than 140 times less than that found in liver, for the same weight of tissue.[24] For $CD4^+$ T cells, migration away from the perivascular space into the parenchyma is inhibited in mice depleted of macrophages, suggesting that there are essential interactions with a perivascular cell after extravasation.[25] The crux of the issue for tumour rejection is whether sufficient T cells reach their target to exert their anti-tumour effect. It is very difficult to quantify this for any tumour, nevertheless, under optimized conditions in different animal models, $CD8^+$ T-cell-dependent immune responses are able to mediate anti-tumour effects in the brain.[6,9,26–33] For spontaneous malignant astrocytoma in humans, the integrity of the BBB is locally compromised, and tumour-induced angiogenesis will not incorporate BBB characteristics. T-cell infiltration frequently occurs,[34–36] but has only occasionally been correlated with a favourable prognosis.[37] However, to date, the specificity and function

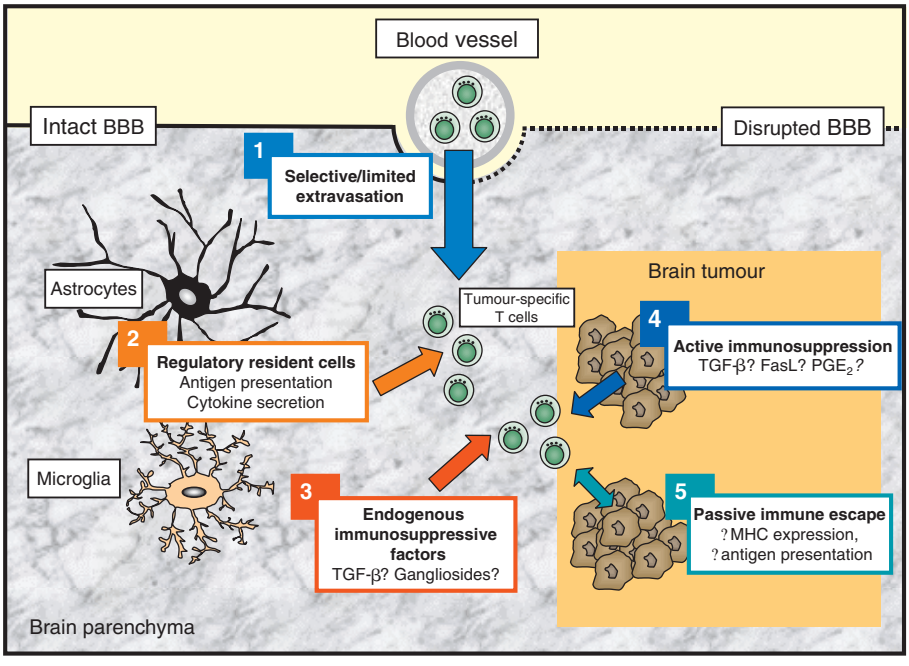

Figure 1. Overview of the obstacles encountered by effector T cells primed in the periphery as they migrate towards a tumour situated in the brain parenchyma. After extravasation through the intact or locally compromised blood–brain barrier (BBB) (1),[1] primed T cells encounter CNS-resident cells such as microglia and astrocytes (2), capable of antigen presentation, tolerance induction, or cytokine-mediated immunomodulation. Constitutive immunosuppressive factors present in the brain parenchyma (3) may also impede full differentiation or expression of effector molecules. As infiltrating T cells reach the tumour bed, they will be exposed to high concentrations of tumour-secreted factors that may synergize with tumour cell surface molecules to down-regulate effector function (4). Finally, efficient immune recognition of the tumour cell may be compromised by passive mechanisms of immune escape, such as low or absent MHC molecule expression and inefficient processing and presentation of tumour-associated antigens (5). Further details and references for these mechanisms are in the text.

of astrocytoma-infiltrating lymphocytes remains to be defined.

Target antigens must permit adequate discrimination of normal and malignant tissue

If we consider the $CD8^+$ cytotoxic T lymphocyte (CTL) as the prime effector T cell for tumour rejection, its fine specificity is of critical importance for the brain. A degree of collateral damage is considered acceptable for tumours in certain extracerebral locations[38] and many defined experimental vaccines aim to induce CTLs that recognize differentiation antigens, such as Melan-A, expressed in melanoma cells and normal melanocytes.[39] Nevertheless, the severity of the autoimmune component of experimental melanoma vaccines, compared with their anti-tumour efficacy is being closely monitored.[40,41] For the CNS, autoimmune reactions in the brain can be very serious, because most tissue is indispensable and has limited capacity for self-renewal. Indeed, early tumour immunology studies noted that lethal allergic encephalomyelitis was induced in different species by immunization with human glioma tissue.[42] It is clear that a better defined tumour vaccine will have a greater chance of avoiding such unacceptable results. However, this necessitates identifying antigens for T cells expressed by brain tumours.

For malignant astrocytomas, identification of antigens recognized by T cells is far less advanced than for melanomas. However, certain antigenic similarities may be expected between melanomas and astrocytomas because their normal tissue counterparts derive from the neuroectoderm. Most studies addressing this possibility have analysed antigen expression at the mRNA level by reverse transcription–polymerase chain reaction. An initial study detected a proportion of tumours expressing one of several melanoma-associated antigens including MAGE family members, tyrosinase, TRP-1, TRP-2, gp100 and p97.[43] However, subsequent publications found a much lower proportion of tumours expressing MAGE antigens, although occasional expression of other cancer-testis antigens was noted (SSX-1, SSX-2, SSX-4, SCP-1, TS85, and MAGE and GAGE family members).[44,45] Although these results underline the potential antigenicity of certain brain tumours, some of the antigens that have a relatively high frequency of expression in malignant astrocytomas may be less useful as targets for immunotherapy because of their high homology to self-antigens (e.g. tumour-expressed GAGE-3 to GAGE-6 and GAGE-8 that are homologous to normal-brain-expressed GAGE-2 and GAGE-7).[45]

Furthermore, the only confirmation that epitopes from any of these antigens are presented at the cell surface for recognition by T cells comes from the study of certain astrocytoma cell lines that can be recognized by MAGE-specific CTL.[46] However, this result reflects MAGE-1 antigen expression in cultured cells, whereas *in vivo*, astrocytomas are generally found to be negative for the MAGE-1 protein.[45,47] This can probably be explained by a different level of DNA methylation induced by culture, because this regulates MAGE expression.[48]

A further candidate astrocytoma antigen that warrants further investigation is $SART1_{259}$, originally identified in epithelial cancer cells and now shown to be expressed in various brain tumour lines and biopsies, including malignant astrocytoma.[49] Astrocytoma cell lines expressing high levels of HLA-A24 and $SART1_{259}$ could be recognized and killed by specific CTL derived from an oesophageal cancer patient, although no evidence of autologous responses was presented. Another possibility for an astrocytoma-associated antigen expression may be from neoepitopes present in epidermal growth factor receptor variant III (EGFR-vIII), expressed in a large proportion of malignant brain tumours.[50] This antigen has until recently been explored as a target for monoclonal antibodies (initially for diagnostic purposes, but more recently for therapeutic application in preclinical mouse models.[51] However, the possibility that EGFR-vIII-encoded T-cell epitopes may contribute to the anti-tumour response has been investigated in mouse models.[52,53] and a vaccine incorporating a peptide from EGFR-vIII is currently under clinical trial.

There must be sufficient MHC molecule expression by tumour cells for direct CTL attack

For most primary brain tumours, the issue of MHC expression is rather different to that in tumours derived from tissues of non-CNS origin, because the normal tissue counterparts of astrocytes and oligodendrocytes are essentially MHC negative or low.[54,55] It is thus essential that MHC molecules are induced either during tumorigenesis or during immunotherapy-induced anti-tumour responses if classical CTL-mediated cytotoxicity is to be operational. For tumour cells of astrocytic origin, these uniformly express MHC class I molecules after *in vitro* culture, and can be induced to express MHC class II after interferon-γ (IFN-γ) treatment (refs 46, 56–58 and our own unpublished observations). The situation *in vivo* is far from clear, with contradictory reports in the literature.[54,55,59,60] This may be because of differences in the immunohistochemistry protocols employed[60] and difficulties in obtaining optimal staining for tumours such as high-grade astrocytomas that are characterized by zones of necrosis.

The generally low MHC expression by normal tissue may actually be an advantage during immunotherapy in that it may spare normal tissue from immune attack. However, normal astrocytes show inducible MHC expression, particularly after incubation with IFN-γ *in vitro*.[56,61–65] *In vivo*, as for neoplastic cells, the situation is more complicated. Based on data from mouse models, some studies have demonstrated that astrocytes can be induced to express MHC class I molecules after viral infection or exposure to IFN-γ.[66–68] However, other authors have suggested that in the absence of degenerated neurones, the oligodendrocyte and the microglial cell are the principal MHC-expressing cells of the non-malignant brain.[69,70] It is clear that this issue of MHC expression of both normal and neoplastic tissue, before and at various stages during treatment, will be important in attempts to measure both positive and any adverse effects for assessment of therapies.

Brain inflammation must be regulated

Uncontrolled inflammation in the CNS rapidly leads to a severe augmentation of intracranial pressure because of the volume limitations within the confines of the skull. This may augment and exacerbate the cerebral oedema caused by the presence of intracerebral tumour mass, compromising neurological function and causing loss of critical cells for which renewal cannot be assured. Furthermore, chronic neuroinflammation may release sequestered autoantigens and contribute to the initiation or perpetuation of autoimmune reactions. Indeed, in a recent rodent gene therapy model for glioblastoma, there was evidence that intracerebral immune responses may have directly or indirectly promoted neuroinflammation and demyelination.[71]

Unfortunately, inflammation is often the accompaniment to vigorous immune responses induced by successful tumour vaccines, with some evidence that this may be occurring (although without mortality) in a mouse model in which there is rapid rejection of an immunogenic tumour implanted intracerebrally.[32] In patients, inflammation is often controlled by administration of steroids, a treatment incompatible with efficacious induction of immune responses. However, there may be a brief and precious window of opportunity for immunotherapy immediately postoperatively whilst tumour burden is minimal and when steroids are not required. Ideally, future immunotherapies will be tailored for the CNS to incorporate limited pro-inflammatory elements. To date, eradication of pre-established, poorly immunogenic intracerebral tumours is still such a major challenge, even in animal models, that there has been little attention paid to ways of minimizing the inflammatory component of the response. Recent advances in understanding how brain-resident cells can contribute to CNS inflammation suggest that successful future immunotherapies will take these local factors into account. For example, based on *in vitro* data, astrocytes are proposed to stimulate more efficiently T helper type 2 (Th2) T cells whereas activated microglial cells, the specialized macrophages of the brain, stimulate a pro-inflammatory Th1 response.[65,72,73] It remains to be determined whether brain-infiltrating lymphocytes *in vivo* are susceptible to Th subset deviation after contact with astrocytes or microglial cells, even if they were primed in secondary lymphoid organs by other antigen-presenting cells (APCs). The wide functional plasticity of reactive (activated) astrocytes is such that various roles are possible,

including a fine tuning of brain inflammation. Thus, depending upon the presence of microenvironmental factors such as IFN-γ or tumour necrosis factor (TNF) and TNF-receptor family members, astrocytes can migrate, release pro-inflammatory cytokines such as interleukin-6 (IL-6) and IL-8, or undergo apoptosis.[74–77] Which of these roles predominates *in vivo* remains to be determined. However, interesting data are emerging from transgenic mouse models in which the glial fibrillary acidic protein (GFAP)-promoter targets expression of various cytokines (IL-3, IL-6, IL-12, IFN-α and TNF) to astrocytes (reviewed in ref. 78). Although such transgenic expression may appear extreme, the levels of transgene-encoded cytokine were similar to those detected in inflamed or infected brains. With age, these mice developed inflammation and degenerative neurological disease.

Anti-tumour effector function must be retained as T cells migrate through the brain parenchyma and encounter resident cells

Although we have described that T cells are able to infiltrate the brain, can effector T cells retain their function as they penetrate the brain parenchyma and approach the tumour mass? Evidence from different models suggests that this will depend upon several factors, such as the magnitude of the induced immune response, the presence of other cell types and the particular subset of the effector cell.

For brain infiltrating T cells, even certain components of normal brain have been suggested to inhibit CTL effector function. For example, T cells otherwise capable of mediating neuropathology were inhibited by brain-derived gangliosides,[79] although this was strain-specific in the mouse, and has not been confirmed in humans. For anti-tumour effector T cells, in the intracerebral P511 mastocytoma tumour model, $CD8^+$ T cells were unable to differentiate into effector cells in the brain microenvironment,[80] a finding that was subsequently attributed to a sensitivity to transforming growth factor-β (TGF-β) present in the cerebrospinal fluid and the interstitial tissue fluid.[81] However, these results were in contrast to those obtained with the similar P815 mastocytoma, which had been transfected with a model antigen, CW3 (P815-CW3): brain-infiltrating lymphocytes tested *ex vivo* from these mice showed full cytotoxic effector function.[32] One difference between these systems was that in the P815-CW3 model, this was a syngeneic response to a defined peptide antigen, whereas in the P511 model most of the experiments were performed in outbred or non-syngeneic mice.

Before the final contact with the tumour cell, the infiltrating T cell may have other encounters with putative APCs of the brain. This has been most clearly defined for MHC class II-restricted $CD4^+$ T cells, although not as yet for cells with specificity for a tumour-expressed antigen. The microglial cell is one of the few MHC class II^+ cells that are resident in the brain and is thus a prime candidate brain APC. As already mentioned, $CD4^+$ T cells require contact with a macrophage-like perivascular cell to advance into the brain parenchyma,[25] however, contact with parenchymally localized microglial cells has been suggested to lead to tolerance induction or the termination of immune responses.[82–85] Such effects were particularly associated with the activation state of the microglial cell.[83] Whether microglial cells are implicated in antigen presentation to $CD8^+$ T cells is not known, however, they may indirectly influence $CD8^+$ T-cell function through their influence on $CD4^+$ T cells. Defined antigen-specific CTL responses in the CNS have principally been studied in antiviral responses that are often strictly $CD4^+$ T-cell-dependent, with loss of cytotoxic function and/or viability in the absence of Th cells.[86,87] However, for anti-tumour immune responses, independence from $CD4^+$ T cells has been demonstrated in several different models.[6,30,32] although it is difficult to generalize because so few studies have looked at this aspect in systems that permit the analysis of specific CTL responses.

The last frontier: retention of effector cell function during the encounter with the tumour cell

The last hurdle to overcome for an anti-tumour effector cell is to function when in intimate contact with the tumour cell. It is at this stage that active mechanisms of tumour immune escape will be most potent: the concentration of soluble immunosuppressive molecules is maximal and may synergize with potential cell-mediated immunosuppressive effects.

There has been a wealth of publications over the past three decades describing immunological defects in astrocytoma patients. These include abnormal delayed hypersensitivity responses, low numbers of circulating T cells, depressed mitogen responsiveness, decreased antibody responses (probably as a result of defective CD4 T helper cell activity) and impaired T-cell cytotoxicity (reviewed by Dix *et al.*[88]). It is reasonable to assume that these defects will be at their most extreme in the vicinity of the tumour, and that they can explain why tumour-infiltrating lymphocytes (TILs) are apparently inefficacious. However, until it can be confirmed that the TILs are really specific for a tumour-expressed antigen, the link remains speculative. Nevertheless, many advances have been made in dissecting the underlying causes for this apparent hyporesponsiveness. It appears that T cells from astrocytoma patients, particularly TILs, express a defective high-affinity IL-2 receptor, or have severe defects in their signalling pathway of activation.[89] Similar findings were also noted in a rat glioma model.[90] This may explain, at least in part, the poor *in vitro* proliferative abilities of T lymphocytes infiltrating malignant astrocytoma, despite the addition of recombinant IL-2,[91] their low IL-2 production after mitogen stimulation,[92] and the difficulty in generating T-cell clones with MHC-restricted cytotoxicity against tumour cells.[93]

Immunosuppression by soluble factors

Soluble factors produced within the tumour microenvironment have long been suspected to be behind many of the above mentioned immune defects. Indeed, T lymphocytes

from normal individuals exhibit similar immunological abnormalities when cultured in the presence of astrocytoma supernatant.[94] The most extensively studied soluble factor is TGF-β_2, originally called glioblastoma cell-derived T-cell suppressor factor, which was first identified in the supernatant of a human glioblastoma cell line that suppressed T-cell growth.[95–97] TGF-β_2 exerts multiple and complex immunosuppressive effects, such as the inhibition of maturation and antigen presentation by dendritic cells or other APCs, inhibition of T-cell activation and differentiation towards effector cells (either cytotoxic cells expressing perforin or Th1 or Th2 cells).[98–100] The role of TGF-β_2 in inducing immunosuppression was further demonstrated in experiments in which anti-sense TGF-β_2 phosphorothioate oligonucleotides inhibited TGF-β_2 secretion by glioblastoma cell lines, restoring the proliferative and cytotoxic functions of autologous lymphocytes.[101] These *in vitro* data naturally encouraged attempts to inhibit TGF-β_2-mediated immunosuppression *in vivo*, which has produced mixed results, probably because TGF-β_2 not only influences immune reactivity, but also acts on the tumour cell directly.[102,103] Other studies, using decorin, a natural inhibitor of TGF-β, suppressed the growth of C6 rat astrocytoma *in vivo*,[104] but these experiments are also complex to interpret because decorin may also be immunostimulatory in a TGF-β_2 independent fashion.[105] Overall, the immunoregulatory functions of TGF-β_2 warrant the attention it has received, but whether it will be feasible or advisable to inhibit this cytokine in brain tumour patients still remains uncertain.

The immunosuppressive properties of malignant astrocytoma-derived supernatants demonstrated *in vitro* cannot be totally accounted for by TGF-β_2. A non-exhaustive list of potentially immunosuppressive molecules detected in astrocytomas or astrocytoma lines includes prostaglandin E_2,[88,96,106,107] gangliosides[108] and IL-10,[109–111] all of which can demonstrate certain immunosuppressive functions *in vitro*. However, whether these factors are playing a major role in inhibiting anti-tumour immune responses *in vivo* is far less certain, either because of doubts that sufficient bioactive factor is released by tumour cells *in vivo* (discussed in ref. 88), or, particularly for IL-10, because *in vivo* function is not necessarily immunosuppressive, but may actually enhance anti-tumour responses.[112,113]

Immunosuppression by cell-mediated interactions

Intercellular contacts through transmembrane molecules such as Fas ligand (FasL) are also proposed to contribute to tumour immune escape. FasL belongs to the TNF family and is implicated in several biological functions through its interaction with Fas, a member of the TNF receptor/nerve growth factor receptor family. FasL–Fas interaction induces the trimerization of Fas and a subsequent complex cascade of intracellular events, potentially leading to apoptosis of Fas-positive cells, a mechanism central to immune homeostasis.[114,115] Although FasL was initially thought to be mainly expressed by cells of haematopoietic origin, it was subsequently shown to be expressed by other normal and neoplastic tissues, including malignant astrocytomas that we have analysed in our laboratory.[116] Expression of FasL in a series of human astrocytoma lines and biopsies was tested, as well as in the rat C6 and mouse MT539MG glioma lines, using a variety of techniques (immunoblot, flow cytometry and reverse transcription–polymerase chain reaction): the majority were positive. Functional data demonstrated that $FasL^+$ astrocytoma cells (cell lines and also astrocytoma cells tested *ex vivo*) can specifically and efficiently kill Fas-transfected P815 target cells. Moreover, an early passage human astrocytoma cell line was able to induce FasL/Fas-mediated apoptosis in $CD4^+$ and $CD8^+$ T cell lines derived from the autologous tumour.[117] It has been suggested that the use of T-cell targets is inappropriate to detect FasL-mediated function by tumour cells, because T cells can also express FasL.[118] However, the kinetics of sensitivity of our T cells to FasL-mediated death are inversely correlated to their endogenous FasL expression; this peaks within the first day after re-stimulation, then rapidly diminishes and is undetectable at the time-points when T cells were tested for Fas sensitivity. Overall these data suggest that astrocytoma cells express functional FasL that can induce apoptosis in Fas^+ targets and that TILs, whilst capable of undergoing apoptosis through fratricide or autocrine suicide, can also be susceptible (at least *in vitro*), of receiving a death signal from FasL-expressing astrocytoma cells. Other groups have independently confirmed expression of FasL by astrocytoma[119–121] and, moreover, apoptotic T cells were observed in the proximity of FasL-expressing astrocytoma cells.[122] However, the real *in vivo* importance of this molecule for astrocytoma, and tumours in general, remains unclear. Indeed, tumour expression of FasL in murine models has been correlated either with enhanced tumour growth[123,124] or with enhanced tumour rejection,[125–127] probably via augmented neutrophil recruitment. Microenvironmental factors clearly influence the consequences of FasL expression by tumour cells, for example, we hypothesized that tumours expressing both FasL and TGF-β may be particularly well adapted to combat CTL effector mechanisms,[117] this principle was subsequently confirmed in an *in vivo* model in which a FasL-positive colon carcinoma could escape rejection if TGF-β was also present.[128] These data help to explain the role of FasL in this particular model (with possible analogy to the situation in the brain), but it is likely that individual combinations of factors relevant to different tumours or models are responsible for the diverse interpretations of these issues in the literature.[117,118,129,130]

Recent additions to the list of astrocytoma-expressed molecules with immunosuppressive potential are HLA-G[131] and CD70,[132,133] although the *in vivo* validation of their function has yet to be reported. HLA-G is a non-classical MHC class I molecule expressed by a limited range of tissues, particularly the placenta, but also certain cancers. Roles in suppressing natural killer and T-cell immune responses have been proposed, but they are controversial.[134,135] Regarding brain tumours, a proportion of astrocytoma cell lines and tumour biopsies expressed HLA-G protein. Functional data showed inhibition of

$CD4^+$ and $CD8^+$ T-cell responses *in vitro*, but this was only tested after incubation of cell lines with high concentrations of IFN-γ (500 U/ml), or after gene transfer of HLA-G into glioma lines. For the transmembrane glycoprotein CD70, previous studies have described expression of this molecule on activated T and B cells, with roles in regulating immune responses via interaction with CD27, expressed on lymphoid cells.[136] Two independent studies have now described expression of CD70 by human glioma cell lines, as well as *in vivo*.[132,133] *In vitro* functional tests suggested a pro-apoptotic role of CD70 when tested on peripheral blood mononuclear cells targets; this was augmented (correlating with expression levels of CD70) when tumour cells were irradiated.[132] However, under certain conditions, T cells shed CD27 after stimulation by $CD70^+$ tumour cells, indicating a possible protection mechanism.

CONCLUDING REMARKS

Will the CNS present an impossible obstacle course for immune effector cells induced during any future brain tumour immunotherapy? There are sufficient encouraging results from certain models to suggest that this will not be the case, because it may not be necessary to overcome all of the mechanisms of immune escape or restrictions imposed by the CNS to achieve some clinical benefit. However, with a better understanding of immune responses in the brain, we can make rational proposals to identify key areas in which to attempt immunomodulation for future brain tumour therapies.

The fundamental importance of understanding immune relations of the brain in health and disease cannot be overstated. It can be assumed that the special features of CNS immune responses, whilst apparently an impediment to the cancer immunotherapist, have evolved to protect this most critical organ of the body. We modify the neuroimmunological balance at our peril. However, the optimal balance that has evolved for a species may not be optimal for an individual. We should therefore not be deterred from shifting the balance towards a protective immune response in patients, as long as the neuropathological risks are understood and monitored.

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