

# Chapter 48

## Harnessing T-Cell Immunity to Target Brain Tumors

Paul R. Walker, Robert M. Prins, Pierre-Yves Dietrich, and Linda M. Liao

**Abstract** T-cell mediated immunotherapy is a conceptually attractive treatment option to envisage for glioma, since T lymphocytes can actively seek out neoplastic cells in the brain, and they have the potential to safely and specifically eliminate tumor. Some antigenic targets on glioma cells are already defined, and we can be optimistic that more will be discovered from progress in T-cell epitope identification and gene expression profiling of brain tumors. In parallel, advances in immunology (regional immunology, neuroimmunology, tumor immunology) now equip us to build upon the results from current immunotherapy trials in which the safety and feasibility of brain tumor immunotherapy have already been confirmed. We can now look to the next phase of immunotherapy, in which we must harness the most promising basic science advances and existing clinical expertise, and apply these to randomized clinical trials to determine the real clinical impact and applicability of these approaches for treating patients with currently incurable malignant brain tumors.

### Contents

48.1	Introductory Remarks .....	1166
48.2	Immune Privilege and Cancer Immunosurveillance .....	1166
48.3	The Stages of Tumor Immunity .....	1167
48.3.1	The Tumor First Stimulates Innate Immune Sentinels, at the Site of the Malignancy .....	1168
48.3.2	The Induction of Adaptive Immune Responses Against Brain Tumors: From the Brain to the Lymph Node .....	1170
48.3.3	The Effector Phase of the T-Cell Mediated Antitumor Response: From the Lymph Node to the Brain .....	1174
48.3.4	The Effector Phase of the T-Cell Mediated Antitumor Response: At the Tumor Site .....	1179
48.4	Glioma Immune Escape .....	1180

---

P.R. Walker (✉)

Centre of Oncology, Geneva University Hospitals and University of Geneva,  
rue Micheli-du-Crest 24, 1211 Geneva 14, Switzerland  
e-mail: Paul.Walker@hcuge.ch

48.4.1	Passive Immune Escape Mechanisms . . . . .	1181
48.4.2	Active Immune Escape . . . . .	1181
48.5	Identification of Glioma-Associated Antigens . . . . .	1184
48.5.1	Identifying Tumor-Associated-Antigens (“Reverse Immunology”) . . . . .	1184
48.5.2	Microarray Technology and Tumor-Associated-Antigens . . . . .	1185
48.6	Preclinical Studies of T-Cell Immunity to Target Brain Tumors . . . . .	1186
48.6.1	Passive Immunotherapy . . . . .	1186
48.6.2	Active Immunotherapy (Tumor Vaccines) . . . . .	1187
48.7	Clinical Trials of Cellular Immunotherapy for Brain Tumors . . . . .	1191
48.7.1	Lymphokine-Activated Killer Cells . . . . .	1191
48.7.2	Cytotoxic T Lymphocytes . . . . .	1191
48.7.3	Dendritic Cell Vaccination Trials . . . . .	1192
48.7.4	Bacterial and Viral Tumor Vaccine Trials for Malignant Glioma . . . . .	1196
48.8	Conclusion . . . . .	1197
	Abbreviations . . . . .	1199
	Glossary of Key Immunological Terms . . . . .	1199
	References . . . . .	1204

## 48.1 Introductory Remarks

Is our immune system capable of recognizing and destroying tumor cells in the brain, either spontaneously or after “doping” by some immunotherapeutic strategy? After some decades of uncertainties concerning tumor immunity and its applicability to the CNS, the answer in the twenty-first century is yes, but with a number of caveats that can best be summarized as “under certain conditions.” The aim of this chapter is to overview the science that may help us to understand the particular circumstances under which elements of the immune system can recognize and destroy tumor in the brain, and then to assess how this information has been put to the test in current clinical trials.

## 48.2 Immune Privilege and Cancer Immunosurveillance

The dialogue between the immune system and brain tumors has been strongly influenced by two key hypotheses, *immune privilege* and *cancer immunosurveillance*, both of which originated more than 50 ago. In the 1940s, Medawar reported that allografts in the CNS survived longer than those in other tissues (Medawar, 1948), which led Barker and Billingham to coin the term *immune privilege* to describe such sites (Barker and Billingham, 1977). The concept of limited immune responsiveness in the brain was interpreted as being consistent with the overriding need to control inflammatory reactions and their potentially damaging consequences to neuronal networks with low regenerative capacity. The absence of a lymphatic system within the CNS and the presence of a specialized blood-brain barrier (BBB) in most CNS compartments suggested immune isolation achieved by restricting entry of blood-born molecules such as immunoglobulins, as well as leukocytes, albeit by mechanisms that are under

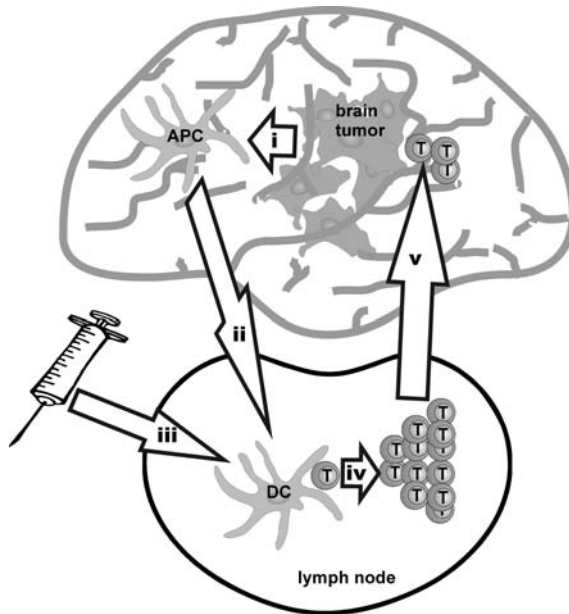
constant revision (Bechmann et al., 2007). With several decades of more detailed observations of immune responses in the CNS, it is now clear that both protective (e.g., antiviral) and pathogenic (e.g., autoimmune) immune responses can and do occur in the CNS, but these may be quantitatively and qualitatively different to those occurring in other sites. In a reappraisal of CNS immune privilege (Bechmann et al., 2007), many compelling arguments were made for a redefinition of privilege, underlining that it is a *relative* state, and only applicable to the parenchyma of the intact, noninflamed brain.

Moreover, perhaps in view of the longstanding, oversimplistic interpretations of immune privilege, one of the cornerstones of modern tumor immunology, the *cancer immunosurveillance hypothesis* of Burnet and Thomas (Burnet, 1970) was not immediately seen to directly concern brain tumors. The theory proposes that the immune system continually surveys the organism and recognizes and destroys abnormal cells. Recognition of cancer certainly occurs, but it is now clear that the outcome of such detection is not always protective immunosurveillance. This led to a refined theory of *immunoediting* (Dunn et al., 2006) in which different outcomes are described following cancer and immune system interaction: namely, elimination, equilibrium, and tumor escape. Analysis of mouse models with clearly defined immunodeficiencies shows that both innate and adaptive immune responses influence tumor outgrowth: mice lacking T lymphocytes, B lymphocytes, natural killer (NK) cells, and those with deficiencies in Type 1 or Type 2 interferons (IFNs) have higher tumor incidence. Moreover, analyses of large cohorts of immunosuppressed patients show that incidence of not only virally associated cancers but also cancers with no known association with infection is increased. However, to date, brain tumors have not been among the tumors noted to occur more frequently. This is likely due to the lower expected frequency of these malignancies, but it may also be because brain tumors are rarely eliminated spontaneously, and more likely persist in equilibrium with the host. Or else they rapidly escape immune control, as would appear to be the case once malignant gliomas are detected. In this case, the notion of immunoediting predicts that outgrowing tumors will have been sculpted by interaction with the immune system, for example, by selection of tumors able to neutralize immune effector mechanisms, as will be discussed (see below).

It should be noted that these ideas are in the context of *spontaneous* immunity against *spontaneous* cancers. When this occurs, and when we can study it, we should do so. Indeed, naturally occurring immune mechanisms endowed by millennia of selection and evolution may provide precious information for rational immunotherapy design.

### 48.3 The Stages of Tumor Immunity

Spontaneous tumor immunity can be conveniently divided into a minimum of three chronologically distinct events (innate immune activation, induction of adaptive immunity, and effector phase of adaptive immunity) that occur in two



**Fig. 48.1** The stages of T-cell mediated tumor immunity. The tissue damage and stress associated with tumor growth will alert cells of the innate immune system, including microglia. Resident or recruited APC (i) phagocytose tumor-derived antigen (e.g., cellular debris) and transport it to LNs (ii). Tumor antigens can also arrive in the LN following peripheral vaccination (iii). Immigrant DC or LN resident DC presents antigen to naïve T cells, leading to their activation and clonal expansion (iv). Activated T cells exit the LN and infiltrate the site of tumor growth (v) to exert their effector functions

distinct anatomical sites: the tumor site and secondary lymphoid tissue (Fig. 48.1). Immunotherapies must reinforce, reproduce, or substitute for each of these phases. We will outline the general features of how and where the stages of an idealized spontaneous antitumor immune response occur, indicating the particularities of the CNS.

### ***48.3.1 The Tumor First Stimulates Innate Immune Sentinels, at the Site of the Malignancy***

#### **48.3.1.1 Detection**

The first step in tumor immunity is alerting the immune system to the presence of tumor; this is achieved by innate immunity. The innate immune system comprises soluble factors such as components of the complement system, but more important for tumor immunity are the cells, such as the macrophages/microglia, granulocytes, NK cells, and dendritic cells (DCs). These cells

efficiently detect pathogen-associated molecular patterns by means of germline-encoded pathogen-recognition receptors (PRRs) that include the toll-like receptors (TLRs). However, their role is now understood to be wider than merely detecting infection by a pathogen; they can also detect perturbations to the organism that can be considered “dangerous” (Matzinger, 1994), and PRR can also be viewed as “pattern” recognition receptors able to deliver danger signals. For example, endogenous ligands have been identified for all human TLRs except for TLRs 5 and 10. These include the heat shock proteins (HSPs) upregulated by stressed cells (binding to TLRs 2 and 4) and nucleic acids released by dying tumor cells (binding to TLRs 3, 7, and 9) (Barrat and Coffman, 2008; Barton and Medzhitov, 2002; Pulendran, 2005).

Detection of cancer will be initially mediated by tissue-resident sentinel cells of the innate immune system, which for most tissues are the DCs and the macrophages. But for the brain, DCs are not resident in the parenchyma (although they may appear at later stages of a response), and the most important endogenous sentinels are the microglial cells (Hanisch and Kettenmann, 2007). These specialized brain macrophages are widely distributed throughout the brain parenchyma and may actually be recruited to the site of glioma occurrence, where they can represent up to a third of the cells composing the tumor (Graeber et al., 2002). Microglia express several TLRs, including TLR 4 that is proposed to bind several factors produced by dying cells in the glioma microenvironment (Hussain et al., 2006). If TLR ligation leads to full activation of the microglial cell, subsequent functions potentially include phagocytosis, chemokine and cytokine release, antigen presentation, and even tumor cell killing. However, other less useful outcomes have been suggested, including neurotoxicity and enhancement of tumor invasiveness through the upregulation of metalloproteinases. These contrasting functions may be attributed to the extreme plasticity of microglia in response to their local milieu. Indeed, the brain microenvironment in which they must function is also comprised of astroglial cells which exhibit characteristics of innate immune function, including PRR expression, activation to a reactive state, and secretion of cytokines and chemokines (Farina et al., 2007). Furthermore, in the case of malignancy, microglial function will also be subject to modulation by the local tumor microenvironment (Hussain et al., 2006; Markovic et al., 2005).

#### **48.3.1.2 Innate Immune Functions**

In a protective antitumor immune response, once the endogenous sentinels of innate immunity are alerted, there are two subsequent events: to limit (or eliminate) the source of danger by innate effector functions of resident cells and to recruit further resources (particularly T cells, as discussed in the next section). For glioma, there is little *in vivo* evidence to suggest that tumoricidal microglia spontaneously restrain brain tumor growth (Graeber et al., 2002). The other innate effector cell that has the potential to recognize and kill tumor cells is the NK cell, which has been the subject of intensive investigations to

characterize the receptors that regulate its function. In order to mediate cytotoxicity, NK cells must receive a signal through one of their activating receptors such as NKG2D, but to result in activation, there must be an absence of signaling through inhibitory receptors (e.g., members of the CD158 family of receptors) (Lanier, 2008). Expression of ligands for NKG2D is low on normal cells, but upregulated on cells subjected to genotoxic stress, as occurs in neoplastic transformation. Indeed, certain human glioma fulfill the requirements for NK cell recognition and activation, with expression of the NKG2D ligands MICA/B (major histocompatibility complex (MHC) class I-chain-related molecules A and B) and UL16-binding proteins (ULBP) 1–3 (Eisele et al., 2006), and a downregulation of some HLA molecules which are ligands for the CD158 inhibitory receptors (Facoetti et al., 2005). NK cells are not only cytotoxic but also an early source of IFN- $\gamma$ , which can be angiostatic and can amplify T-cell mediated tumor immunity. However, little is known about the NK cells or their potential impact at the tumor site in human glioma; although in a murine intracranial tumor model (B16 melanoma), interactions between DCs, NK cells, and T cells facilitated protective tumor immunity (Prins et al., 2006c).

### ***48.3.2 The Induction of Adaptive Immune Responses Against Brain Tumors: From the Brain to the Lymph Node***

Although macrophages and microglia are the major components of the immune infiltrate in brain tumors at every stage of the response, after initial detection of the malignancy they will be joined by T lymphocytes, which are responsible for the cell-mediated immune responses of adaptive immunity. The B lymphocytes, giving rise to antibody-secreting plasma cells and the humoral arm of adaptive immunity, are probably not a major component of spontaneous immunity to brain tumors. On the other hand, antibodies administered therapeutically have important applications in glioma therapy and will be covered elsewhere in this book (see Chapter 36). In this chapter, the focus is on T cells (both CD4 and CD8 subsets), which once activated have the potential to infiltrate tumors and mediate potent effector functions including cytotoxicity and local cytokine release. It will become clear that while these potentially useful effector functions may be T-cell mediated, their initiation is totally dependent on innate immune cooperation.

#### **48.3.2.1 Generation of Naïve CD4 and CD8 T Cells**

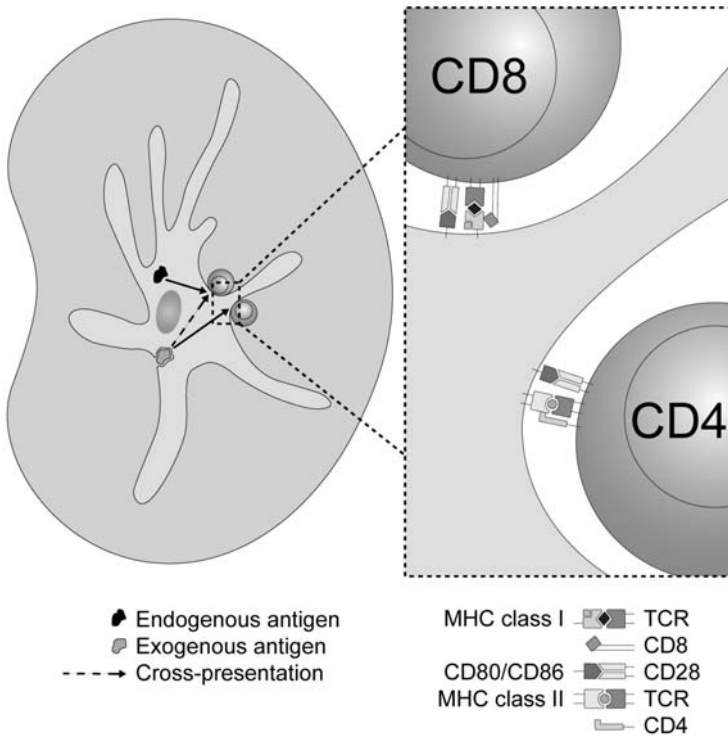
T cells that are mature and naïve (i.e., cells not yet stimulated by their cognate antigen) are generated in the thymus, and then travel in the blood and lymph to recirculate between secondary lymphoid organs such as the spleen and LNs. Although the majority of naïve T cells do not efficiently enter nonlymphoid

tissues, including the CNS, exceptions have been reported (Brabb et al., 2000). Such atypical T-cell trafficking probably does not account for a significant proportion of T cells under normal conditions and may even be associated with tolerance induction rather than induction of efficient antitumor immunity. Therefore, for understanding the generation of efficacious tumor immunity, the “conventional” trafficking and activation of T cells in LNs will be considered here.

A large number of T cells are generated in each individual, with each bearing clonally distributed antigen receptors that are generated by somatic rearrangement of a limited number of gene segments. These encode a heterodimeric T-cell receptor for antigen (TCR) comprised of  $\alpha$  and  $\beta$  chains for the main T-cell population that will be discussed here, and of  $\gamma$  and  $\delta$  chains for receptors used by a minority  $\gamma\delta$  T-cell population. The ligands for the TCR of CD8 T cells are short peptides of around 8–10 amino acids bound to class I MHC molecules, whereas CD4 T cells recognize slightly longer peptides ( $\sim$ 13–17 amino acids) bound to MHC class II molecules. Since TCR are randomly generated, the repertoire potentially includes receptors of any conceivable specificity, but the frequencies of naïve T cells expressing a TCR of a given specificity in the whole T cell population are very low (in the order of 1 in  $10^6$  cells), necessitating significant clonal expansion in order for T cells to achieve a biological effect *in vivo*.

#### 48.3.2.2 Naïve T-Cell Activation Requires Two Signals

If naïve T cells receive only a single signal via ligation of their TCR by an MHC/peptide complex, they may become anergic, *i.e.*, without effector function and refractory to further stimulation. Full activation of naïve T cells to become effector cells requires a second signal, which is usually delivered by costimulatory molecules such as members of the B7 family (particularly CD80 and CD86) expressed by antigen presenting cells (APCs) of the innate immune system, the most efficient of which are the DCs (Fig. 48.2). Activation of naïve T cells (priming) by APCs within the secondary lymphoid tissue triggers clonal expansion of T cells and a differentiation program that will influence the magnitude and quality of the antitumor immune response, *i.e.*, T-cell effector functions (cytotoxicity, cytokine release, suppression), tissue tropism, and persistence as T effector or T memory cells. Although this is occurring in the LN, these factors are principally predetermined by the innate immune reaction, upstream at the tumor site (or at a vaccination site). The resulting clonally expanded T cells (now highly enriched for tumor antigen specificity) can then exit the LN via the efferent lymph, enter the bloodstream, and traffic more or less efficiently to the tumor site, depending upon the pattern of adhesion molecules and chemokine receptors (their “homing phenotype”) with which they have been programmed to express by the DC.



**Fig. 48.2 T-cell activation in the lymph node.** Naïve T cells are activated (primed) in secondary lymphoid tissue by DCs that are able to present tumor antigens (i.e., exogenous antigens) on MHC class I (cross-presentation) and MHC class II molecules to CD8 and CD4 T cells, respectively. In addition, all DCs present endogenous antigens to CD8 T cells. The DCs also express costimulatory molecules (e.g., CD80/CD86) that deliver a second signal to receptors on the T cells, ensuring T-cell activation rather than tolerance (see text)

**48.3.2.3 How Does Antigen from the Tumor Site Reach the Naïve T Cells in the Lymph Node?**

The best understood route for antigen transport to the LN is via DCs, which are able to phagocytose tumor-derived antigens, then migrate via lymphatic vessels to the T-cell zones of draining LN, wherein they can await encounter with a recirculating naïve T cell with specificity for the captured antigen bound to an MHC molecule. For brain tumors, this scenario is complicated by the particularities of CNS cellular composition and anatomy: there are no lymphatic vessels in the brain, and there are no resident DCs in the healthy brain parenchyma. Nevertheless, several different experimental approaches all now confirm that functional antigen drainage from the brain parenchyma to lymphoid tissues occurs, principally to the cervical LNs (Cserr and Knopf, 1992).



#### 48.3.2.4 Cell-Free Drainage of Antigen

Early studies examined transport of soluble products which flow with interstitial tissue fluid via the perivascular spaces, to drain either into the subarachnoid space or to lymphatics via arachnoid sheaths of certain cranial nerves and spinal nerve roots to reach the cervical LNs (Bechmann et al., 2007). More recently, particulate material was also shown to reach cervical and submandibular LN after intracerebral injection (Walter and Albert, 2007). These studies confirm functional drainage in the absence of lymphatics, but they do not adequately explain the initiation of adaptive immunity. For this to occur without induction of immune tolerance, there must be not only antigen presented to T cells in the cervical LN but also a second signal indicating danger or infection. The best understood source of such a second signal is an APC that has sensed danger at its source, i.e., at the tumor site in the brain.

#### 48.3.2.5 Cell-Mediated Transport of Antigen from the Brain to the Lymph Node

A cell able to transport brain tumor-derived antigenic material to LNs and stimulate T cells therein should have the following properties: it should be phagocytic; it should be able to process and present tumor antigens on MHC class I and class II molecules; it should express costimulatory molecules; and it should be migratory. The microglia are the most abundant phagocytes in the brain, and they can be induced to express costimulatory molecules, and they can present antigens to MHC class II restricted CD4 T cells in vitro (Aloisi et al., 2000). The more demanding function of cross-presentation, i.e., uptake of exogenous antigens and presentation of processed peptides derived therefrom on MHC class I molecules, has only been reported in one in vitro study using ovalbumin as antigen (Beauvillain et al., 2008). Whether these in vitro results apply to microglia in vivo is debatable, partly because of the criteria used to define microglia, such as morphology, anatomic location, and level of expression of macrophage associated markers, and even function (Davoust et al., 2008). Indeed, efficient antigen presentation in vitro requires a phenotype and function of a reactive, or activated microglial cell, which is virtually indistinguishable from any activated macrophage. The final function necessary for in vivo initiation of T-cell immunity is migration to the LN. Expression of the chemokine receptor CCR7 is associated with migration to LN and has been reported for murine microglia (Dijkstra et al., 2006), but functional LN homing experiments were not performed.

In vivo studies suggest that brain APC function for presentation of peptides to CD4 T cells resides in a population of perivascular cells originally characterized by their radiosensitivity (Platten and Steinman, 2005). In fact, these cells may represent “differentiated” microglia that can express the DC marker CD11c and that morphologically resemble DCs (Fischer and Reichmann, 2001). However, phenotypic studies do not always allow extrapolation to function, and in different mouse models, CNS DC interactions with T cells have been defined as either stimulatory (Fischer and Reichmann, 2001; Ling et al., 2008) or inhibitory (Suter

et al., 2003). Overall, these studies confirm that the plasticity of microglial cells may give rise to cells that resemble macrophages or DCs, but they do not give definitive answers for the origins and identity of the cell that is ultimately able to form the link between innate immune detection of malignancy in the brain and the induction of adaptive immunity in the LN. This could still be a conventional DC or a precursor recruited either from peripheral brain regions rich in CD11c-expressing cells (choroid plexus, meninges) or from venous blood in response to initial microglial and astroglial activation.

An alternative approach to address the issue of CNS to LN APC migration has been to inject labeled DCs in the brain and track their subsequent migration. Such approaches have in some cases confirmed migration to cervical LNs (Carson et al., 1999; Ehtesham et al., 2003; Karman et al., 2004; Kuwashima et al., 2005), although there may be limitations to the interpretation of such systems. First, the migratory behavior of injected DCs may not accurately reflect endogenous pathways, and second, the volume and site of injection within the CNS (brain parenchyma vs. ventricles) may influence the migration pattern (Bechmann et al., 2007; Hatterer et al., 2006; Thomas et al., 2008). However, despite these considerations, T-cell activation and expansion is observed in the cervical LNs in brain tumor models reliant on endogenous APCs (Calzascia et al., 2005; Prins et al., 2008b; Walter and Albert, 2007).

### ***48.3.3 The Effector Phase of the T-Cell Mediated Antitumor Response: From the Lymph Node to the Brain***

Activated T cells enriched for tumor specificity can be primed in various ways: in a spontaneous immune response, after vaccination, or even in vitro. But in all cases, to impact on the tumor, they need to enter the site harboring the malignancy, infiltrate the tumor bed, and exert their effector functions in the local tumor microenvironment. Although certain general properties of effector T cells apply to any tumor in any site, optimal antitumor function in the context of a cerebral malignancy requires specific features in the T-cell response.

#### **48.3.3.1 T-Cell Entry to the Brain and Antigen Specificity**

Most reports suggest that the principle factors influencing entry of T cells to the brain are the activation or differentiation status of the T cell, and the activation status of the vasculature. Nevertheless, an antigen-specific component of CD8 T-cell recruitment to brain was proposed in a study in which peptide was injected intracerebrally, then subsequently presented on the luminal surface of endothelial cells (Galea et al., 2007). However, it is currently unclear whether endothelial cells would be as efficient in phagocytosing and cross-presenting tumor-derived antigens as they are in presenting soluble peptide, and so it remains to be determined whether such a mechanism will have relevance for tumor immunity. Generally,

there are considered to be more opportunities for T cells to interact with their specific antigen once they traverse the endothelium barrier, as demonstrated by the preferential retention in the brain of autoreactive and virus-specific CD4 T cells (Hickey, 1999) and of tumor specific CD8 T cells (Calzascia et al., 2003).

### 48.3.3.2 Antigen-Independent T-Cell Extravasation to the Brain

General features of leukocyte extravasation are common to all tissues because of similar hemodynamic constraints. The enormous velocity of the T cell in the bloodstream relative to the static endothelial cell forming the vessel structure is initially reduced by transient adhesive interactions between the T cell and the endothelium. Selectins (e.g., E-selectin, P-selectin) on the endothelium engage their ligands on T cells, slowing the flow of the T cells so that they begin to roll along the vessel wall. This facilitates contact between chemokine receptors on the T cell (such as CCR5) and corresponding immobilized chemokines on the luminal face of the endothelial cell (such as CCL5). Chemokine receptor signaling leads to conformational changes in T-cell-expressed integrins, allowing firm adhesion to the cell adhesion molecules on the endothelium, arrest, and diapedesis (Butcher et al., 1999). Tissue-specific combinations of adhesion molecules and chemokines allow selective recruitment of particular T-cell populations (Luster et al., 2005).

Many of the underlying concepts of immune cell entry into the CNS are based on CNS autoimmune conditions, principally multiple sclerosis (MS) in patients and experimental autoimmune encephalomyelitis (EAE) in mice (Engelhardt, 2006). This precious information may also apply to cerebral malignancies but will require validation. Indeed, there is a preponderance of information on CD4 T cells in EAE, but less on CD8 T cells, and furthermore, certain molecular considerations of homing are proposed to be influenced by the mouse strain, and the precise CNS region, factors that have not yet been addressed for tumors. Nevertheless, several key findings do now appear to apply to different strains, species, and neuropathologies (Table 48.1).

**Table 48.1** Key adhesion molecules and chemokine receptors proposed for T-cell extravasation to the CNS

Receptor/molecule on T cell	Ligand/counterreceptor
<b>Adhesion molecules*</b>	
LFA-1 ( $\alpha_1\beta_2$ integrin, CD11a/CD18)	ICAM-1, ICAM-2, JAM-A
$\alpha_4\beta_1$ integrin (VLA-4, CD49d/CD29)	VCAM-1, JAM-B
E- and P-selectin ligands (several, contain sialo-fucosylated Lewis carbohydrates)	E-selectin, P-selectin
CD6	ALCAM (CD166)
<b>Chemokine receptors*</b>	
CXCR3	CXCL9, CXCL10, CXCL11
CCR5	CCL3, CCL4, CCL5, CCL8, CCL3L1
CCR7	CCL19, CCL21

\*Non-exhaustive listing, referring only to molecules discussed in text.

### 48.3.3.3 Role of Integrins in CNS Tropism

The adhesion molecule that is consistently implicated in T-cell entry to the CNS in most species and models tested is  $\alpha_4$  integrin (CD49d). Integrins are expressed as heterodimers and  $\alpha_4$  partners with either  $\beta_1$  (CD29) or  $\beta_7$  integrin chains. For the CNS, the  $\alpha_4\beta_1$  integrin (also called VLA-4) and its interaction with VCAM-1 on endothelial cells are central. In EAE,  $\alpha_4\beta_1$  may facilitate both lower affinity rolling interactions and higher affinity arrest, according to the particular CNS microvasculature involved (Engelhardt, 2006). In mouse brain tumor models,  $\alpha_4\beta_1$  integrin was also shown to be a key molecule for T-cell entry to the tumor site. We found that high levels of  $\alpha_4\beta_1$  integrin are induced on tumor-specific CD4 T cells (PRW and PYD unpublished data) and CD8 T cells (Calzascia et al., 2005), when they are primed by endogenous APC in the cervical LN of brain tumor-bearing mice. This was functionally relevant since  $\alpha_4$ -specific blocking antibody significantly reduced brain entry of these T cells. Further exploration of the role of integrins in brain tropism has been performed on CD8 T cells polarized toward different cytokine-secreting profiles. Mouse CD8 T cells with a type 1 profile (IFN- $\gamma$ -secreting) expressed higher levels of  $\alpha_4\beta_1$  integrin and trafficked to the brain more efficiently than type 2, interleukin (IL)-4 secreting CD8 T cells (Sasaki et al., 2007). This finding is particularly relevant for brain tumor immunotherapy, since T cells receiving the same in vitro or in vivo stimuli can manifest two key characteristics for efficacious antitumor function: appropriate tissue tropism and cytokine expression. The same key  $\alpha_4\beta_1$  integrin is also implicated in CNS entry of T cells in humans. Patients with MS receiving a novel treatment in which  $\alpha_4$  integrins were targeted using the humanized antibody natalizumab had reduced levels of inflammatory leukocytes in the cerebrospinal fluid, and generally showed some clinical improvement (Stuve et al., 2006). Taken together, the results from these different studies unequivocally establish an important role for  $\alpha_4\beta_1$  integrin in T-cell homing to the CNS. However, this should not be interpreted to mean that  $\alpha_4\beta_1$  specifically targets T cells to the brain, since most of the studies have analyzed a single tissue, the brain. Rather, it is likely that if  $\alpha_4\beta_1$  integrin is abundantly expressed by a T cell; this molecule can facilitate entry to the CNS. Nevertheless, since the inhibition or blocking of  $\alpha_4\beta_1$  does not totally abrogate T cell entry to the CNS, other molecules are probably also involved and may either substitute for or synergize with  $\alpha_4\beta_1$ .

### 48.3.3.4 Role of Non-integrin Adhesion Molecules

To date, there are mixed findings for the involvement of non-integrin adhesion molecules in T-cell entry to the CNS. Nearly all of this data derives from studies on EAE and MS, with findings that are often specific for particular CNS regions and specific stages of the disease. Roles in T-cell extravasation to brain tumors, either in spontaneous immunity or in immunotherapy applications, cannot be excluded. An induction of E- and P-selectin ligands on tumor-specific CD8 T cells

dividing in the cervical LN was observed in mice harboring intracranial tumors, but the functional significance of this observation was not analyzed (Calzascia et al., 2005). P-selectin (and to a lesser extent, E-selectin) and their ligands were found to contribute to T-cell rolling on certain inflamed CNS microvessels using intravital microscopy and *in vitro* studies. This study concluded that CD8 T cells from MS patients preferentially rolled via P-selectin, whereas CD4 T cells rolled via  $\alpha_4$  integrin (Battistini et al., 2003). However, others have found that CNS inflammation was not altered following genetic ablation or overexpression of E- and P-selectins or their ligands, or after antibody blocking (Engelhardt, 2006). Therefore, the real *in vivo* significance of the selectins, at least in EAE, has been difficult to establish.

A further adhesion molecule that may influence T-cell adhesion to endothelium is lymphocyte function-associated antigen-1 (LFA-1), which is expressed by naïve and activated T cells. Intercellular adhesion molecules 1 and 2 (ICAM-1, ICAM-2) are the ligands for LFA-1 and are present either constitutively or after activation on CNS microvessels, including those of human glioma (Engelhardt, 2006; Kuppper et al., 1990). *In vitro* studies in EAE suggested potential roles for LFA-1 particularly during the transendothelial migration of T cells. *In vivo* experiments have been difficult to interpret, probably because LFA-1 and ICAM interactions are also critical for the immunological synapse that facilitates the antigen-specific interactions between T cells and APC or target cells.

A recent observation highlighted the role of activated leukocyte cell adhesion molecule (ALCAM) in facilitating CNS infiltration of T cells and monocytes (Cayrol et al., 2008). Expression of ALCAM was noted to be higher on BBB endothelial cells than endothelium from other organs, and it was upregulated in inflamed vasculature. Antibody blockade experiments showed that CD4 T cell and monocyte transmigration across brain endothelial cells was partially ALCAM dependent, and ALCAM neutralization *in vivo* reduced EAE severity. It was noteworthy that among the T cells, only the CD4 T-cell subset appears to use ALCAM during transmigration, since CD8 T cells were totally unaffected, even though they express similar levels of the CD6 ALCAM receptor. These findings, if confirmed in the context of brain tumor immunity, may open up future possibilities to modulate T-cell subset brain infiltration in future immunotherapies.

#### 48.3.3.5 Role of Chemokines and Chemokine Receptors

Chemokines are low molecular weight chemoattractant cytokines that function by binding to G-protein coupled receptors expressed on a wide range of cells, including leukocytes. Unraveling the roles of individual chemokines (~50) and their receptors (~20) is complex because chemokines can bind multiple receptors, and receptors can bind multiple chemokines. Furthermore, in the context of brain tumors, the tumor itself is a source of chemokines which may influence the quantity and quality of the resulting immune infiltrate (Van Meir, 1999, Dey et al., 2006, Brown et al., 2007; Jordan et al., 2008). Chemokines are proposed to

influence multiple steps of CNS infiltration, from the blood to migration within the brain parenchyma, although not all steps have been validated *in vivo* in the CNS (Rebenko-Moll et al., 2006). In the lumen of CNS microvessels, chemokines immobilized on endothelial cells can trigger integrin activation of tethered leukocytes (particularly  $\alpha_4\beta_1$  in the context of brain tropic T cells) and facilitate high-affinity interactions and arrest. Diapedesis can then be promoted by chemokines mediating locomotion to interendothelial junctions. T cells may then sample abluminal chemokines by extending processes through intercellular junctions. And finally, chemokine gradients exist within the brain parenchyma to attract T cells to the tumor site.

Concerning the role of specific chemokines in T-cell infiltration of brain tumors, very few *in vivo* studies have been performed, and conclusions are mostly based on EAE, MS, or infection with neurotropic viruses (Rebenko-Moll et al., 2006). The proportion of T cells expressing CXCR3 is enhanced among T cells present in the cerebrospinal fluid of patients with MS and in EAE, and the levels of two of its ligands, CXCL9 and CXCL10, are elevated in the CSF during acute phase of MS. However, *in vivo* experiments with blocking antibodies and CXCR3 deficient mice have yielded conflicting results, probably because CXCR3 influences EAE at multiple levels (e.g., IFN- $\gamma$  production) and not just at the level of trafficking. Similar considerations exist for CCR5 and its ligand CCL5, which are suggested to have roles in CNS viral infection (including West Nile virus, HIV, and coronavirus) but probably relate to T-cell function rather than trafficking. Another chemokine receptor expressed on T cells, CCR7, and its ligands CCL19 and CCL21 may have multiple roles in CNS pathologies. The cerebrospinal fluid of patients with MS and brain lesions of mice with progressive EAE accumulate CCR7-expressing T cells. Moreover, in addition, two ligands of CCR7, CCL19 and CCL21, are expressed at the BBB in brain of mice with EAE and at least *in vitro*, can mediate adhesion of CCR7<sup>+</sup> T cells. However, studying the *in vivo* functional significance of this is difficult when using blocking antibodies or gene deficient mice, since naive T cells, a subset of memory T cells (T<sub>cm</sub>), as well as some DCs, all express CCR7.

#### **48.3.3.6 Suboptimal Trafficking of T Cells to Brain Tumors May Lead to Suboptimal Tumor Therapies**

As the previous sections indicate, there is little direct data about the properties of protective T cells able to traffic efficiently to brain tumors. Nevertheless, the level of infiltration of tumor-specific T cells will be a factor that will limit the efficacy of immunotherapies. Many current tumor T-cell immunotherapy protocols are adapted directly from therapies being tested for tumors in other sites, and yet the tumor pathology is fundamentally different. The lethality of most extracranial tumors is due to metastases, and the goal of T-cell immunotherapy will be that therapeutic T cells can reach the many sites of tumor dissemination. For primary CNS malignancies such as malignant glioma, the situation is different; these tumors are unique in oncology because they very rarely

metastasize from their tissue of origin. The immunological problem for such tumors is thus strictly one of regional immunity. Thus, promoting efficient brain tropism of *protective* immune cells will undoubtedly benefit future glioma immunotherapies. For this it will be necessary to not only understand and induce the key molecules for T cell-entry to the CNS but also avoid induction of a homing phenotype that may lead to their entrapment elsewhere (for example, the mucosal surfaces if there is high  $\alpha_4\beta_7$  and CCR9 expression). In this regard, preclinical models in which multiple sites are studied will be helpful (Calzascia et al., 2005), for which the emerging technology of whole body imaging will be particularly appropriate (Prins et al., 2008b). A further refinement would be to ensure infiltration of only protective T cells, since a gross augmentation of any inflammatory infiltrate will be inappropriate and dangerous for the brain. An understanding of how subset-specific trafficking can be achieved would be a useful objective in the optimization of therapies.

### ***48.3.4 The Effector Phase of the T-Cell Mediated Antitumor Response: At the Tumor Site***

#### **48.3.4.1 CD8 T Cells**

In an idealized antitumor immune response, tumor-specific CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs) that have penetrated the brain parenchyma and made contact with the tumor will kill malignant cells by direct cell-mediated cytotoxicity. This occurs in some preclinical brain tumor models, and to date, possibly for a minority of malignant cells forming the intracranial tumor mass in patients with glioma. If the CD8 T cell has been fully activated in the periphery, the tumor cell needs only to express MHC class I/peptide (costimulatory molecules are not essential at the effector stage) for it to become a CTL target. Cytotoxicity occurs by the polarized exocytosis of the contents of cytotoxic granules into the immunological synapse formed between the CTL and the tumor target, and it is exquisitely specific, with no bystander killing. The granule contents include perforin and granzymes that perturb the tumor cell membrane and serine proteases known as granzymes that induce tumor cell death mainly through caspase-dependent pathways. Secondary cytotoxic mechanisms also exist, through cell-associated or cell-secreted cytotoxic molecules, including Fas ligand (CD95L), tumor necrosis factor (TNF), and lymphotoxins (LTs).

Antitumor effects can also be mediated by CD8 T cells after indirect recognition of tumor derived antigenic peptides on MHC class I molecules of an APC, i.e., by cross-presentation. As already discussed, the role of APCs in priming CD8 T-cell responses in the LN is essential, but they may also have a role at the effector stage of the response. Indeed, local APCs can potentially amplify T-cell-mediated antitumor effects occurring at a low level after direct T cell-tumor cell contact (Karman et al., 2006; Masson et al., 2007), or they may

substitute for this contact in the absence of direct antigen presentation by the tumor cell, for example, because of MHC downregulation. One of the key factors proposed for an indirect antitumor effect is IFN- $\gamma$ , which impedes tumor growth by acting on IFN- $\gamma$  receptor expressing tumor stroma and inhibiting angiogenesis (Qin et al., 2003).

Cross-presentation of antigen to CD8 T cells has been demonstrated *in vitro* by microglial cells (Beauvillain et al., 2008), but the principle cross-presenting APC *in vivo* is most likely to be the DC (Jung et al., 2002). Cross-presenting DCs in the brain have not yet been reported, to our knowledge, for human malignant glioma. One consequence of cross-presentation of antigen is that antigen-specific T cells will be retained at the site of this cellular interaction. Indeed, we observed retention of tumor-specific CD8 T cells in the brain in a mouse model designed to address the issue of cross-presentation (Calzascia et al., 2003). In this model, intracranially implanted MT539MG astrocytoma cells were unable to directly present a defined tumor antigen, and so the presentation of this tumor antigen to T cells in the brain was occurring solely through cross-presentation, although the identity of the APC in this study was not determined. In several other experimental situations, indirect stimulation of CD8 T cells at the tumor site has been achieved by intracranial injection of DCs (Ehtesham et al., 2003; Kikuchi et al., 2002; Masson et al., 2007; Nishimura et al., 2006; Pellegatta et al., 2006). The local functions of these cells may be to enhance T-cell proliferation at the tumor site, promote T-cell retention in the brain, and/or to amplify effector functions such as IFN- $\gamma$  release.

#### 48.3.4.2 CD4 T Cells

The role of CD4 T cells in brain tumor immunity is complex, because CD4 T cells can differentiate toward at least four different subsets ( $T_{h1}$ ,  $T_{h2}$ ,  $T_{h17}$ , and  $T_{reg}$ ) that are difficult to identify phenotypically and that can have either pro- or antitumor effects. Moreover, very few (if any) glioma antigens recognized by CD4 T cells have been identified. Nevertheless, it is assumed that an IFN- $\gamma$ -secreting CD4  $T_{h1}$  cell component of brain tumor immunity may have antitumor activity and potentially aid CD8 T-cell accumulation, survival, and function, as has been demonstrated in certain (but not all) rodent models (Ciesielski et al., 2008; Wang et al., 2007).

### 48.4 Glioma Immune Escape

The inevitable progressive growth of malignant glioma indicates that the idealized T-cell mediated antitumor response does not occur spontaneously or that it is neutralized by the time high-grade gliomas are clinically detectable. There are a multitude of passive and active immune escape mechanisms that are proposed to explain this and which may have contributed to the impaired



cellular immune function in glioma patients that has been reported for decades (Walker et al., 2003). However, these are putative mechanisms that have not yet been validated in vivo for malignant glioma in patients, with the exception of transforming growth factor (TGF)- $\beta$ , as will be discussed. Arguably, what may be equally important is the low-level induction of spontaneous antitumor immunity and the non-immunological treatments (radiotherapy, chemotherapy, steroidal anti-inflammatory drugs) that may antagonize any nascent protective immune response.

#### ***48.4.1 Passive Immune Escape Mechanisms***

Gliomas may attempt to passively escape immune detection by downregulation of MHC expression or of molecules associated with antigen presentation to CD8 T cells. Interestingly, these characteristics are associated with higher grade astrocytomas (Facoetti et al., 2005; Mehling et al., 2007). Of importance for future immunotherapy strategies are the in vitro observations that MHC can be upregulated on glioblastoma cell lines by IFN- $\gamma$  or IFN- $\alpha$  (Yang et al., 2004). Tumor cells escaping T-cell mediated immunity by downregulating MHC expression may risk attack by NK cells, which are normally inactive if their inhibitory receptors are ligated by MHC. However, several adaptations of malignant glioma may guard against this. Expression of MICA and ULBP2, ligands for the NKG2D activating receptors on NK cells, was low or absent for WHO grade III and IV astrocytomas (Eisele et al., 2006), whereas an array of ligands (HLA-E, HLA-G, lectin-like transcript-1) for inhibitory NK receptors was overexpressed (Roth et al., 2007; Wischhusen et al., 2007). A further major consideration that can be considered as a factor leading to immune escape is the presence of areas of hypoxia, well documented in human glioma (Louis, 2006). These areas of tumor will be a particularly hostile microenvironment for immune cells, and the function and survival of T cells may be particularly sensitive to low oxygen tension (Sitkovsky and Lukashev, 2005).

#### ***48.4.2 Active Immune Escape***

Caution should be exercised in interpreting the role of “immunosuppressive” molecules. Immunosuppressive effects have often been established in vitro or in some cases in vivo with ectopic overexpression of the molecule under test. The ultimate in vivo role will depend upon the microenvironmental context, and the level of expression, which may be very different in the human pathology and in animal models. Much ingenuity will be needed to determine whether these molecules will really influence human glioma pathogenesis and response to treatment, and in the meantime, our conclusions must remain provisional.

#### 48.4.2.1 Soluble Immunosuppressive Molecules

Soluble factors can act either directly on the effector T cell or through recruitment of a third party “immunosuppressive” cell. In some cases, the same molecule may function in multiple ways, the most notorious example of which is TGF- $\beta$ . This multifunctional cytokine not only directly suppresses NK and T-cell proliferation and antitumor functions (including granzyme, FasL and IFN- $\gamma$  expression) but also promotes other suppressive cells (see below), angiogenesis, and tumor invasion (Wrzesinski et al., 2007). TGF- $\beta$  (and in particular the TGF- $\beta_2$  isoform) is produced by glioma cell lines and by glioblastoma in vivo (Bodmer et al., 1989; Liau et al., 2005). Moreover, in a phase I DC vaccination trial for glioblastoma, we noted increased intratumoral infiltration by CTLs in four of eight patients who underwent reoperation after vaccination, which was inversely correlated with TGF- $\beta_2$  expression within the tumor and positively correlated with clinical survival ( $P = 0.047$ ) (Liau et al., 2005). Consequently, TGF- $\beta$  or its effects have become attractive targets, and novel therapeutic approaches with inhibitors and antisense oligonucleotides are being actively explored. Other soluble factors of astrocytoma origin that may have immunosuppressive potential include prostaglandin E<sub>2</sub>, gangliosides, and IL-10 (Walker et al., 2003), but the real in vivo concentration and impact of these factors has yet to be firmly established.

#### 48.4.2.2 Cell Surface Immunosuppressive Factors

A candidate immunosuppressive molecule is Fas ligand (CD95L), which is involved in immune homeostasis and cytotoxicity by inducing apoptosis in target cells. Gliomas express Fas ligand in vitro and in vivo. In vitro, Fas ligand expressing glioma cell lines can kill Fas (CD95) expressing CD4 and CD8 T cell lines from the autologous donor, but in vivo, the role of Fas ligand expressing tumor cells is controversial (Walker et al., 2003, 1997). Gliomas also express B7-H1 (also called PD-L1), a member of the B7 family that is able to interact with T-cell expressed programmed death-1 (PD-1) receptor expressed by T cells (Wilmotte et al., 2005; Winterle et al., 2003), an interaction which negatively regulates T-cell activation. Interestingly, the expression of B7-H1 has been associated with a genetic event in gliomas, the loss of the tumor suppressor PTEN, which activates the PI3 kinase pathway (Parsa et al., 2007). The immunosuppressive potential of PD-1-B7-H1 interactions in vivo has yet to be explored in intracranial tumors. CD70 is another cell surface molecule proposed to facilitate glioma immune escape as it can engage the counterreceptor on immune cells and induce apoptosis. CD70 is expressed by glioma in vitro and can indeed trigger apoptosis of immune cells (reviewed by (Walker et al., 2003)). However, the first in vivo studies of gliomas transfected with CD70 indicated an immune stimulatory role, and the authors even suggested exploiting the CD70–CD27 axis in immunotherapy (Aulwurm et al., 2006). The role of CD70 expressed by non-manipulated human glioma in vivo therefore awaits clarification.

### 48.4.2.3 Immunosuppressive Cells

In recent years, there has been a major resurgence in the interest and understanding of cellular-based mechanisms that may regulate or suppress tumor immunity. Cells of different origin (see below) can be involved in suppressing immune responses and have valuable roles in regulating autoimmunity and controlling inflammation. However, these functions may also blunt spontaneous or vaccine-induced tumor immunity. Furthermore, immunosuppressive cells can in some cases be recruited or induced by factors (e.g., cytokines such as TGF- $\beta$ ) produced by tumor cells. Mesenchymal stem cells have strong anti-inflammatory or antiproliferative effects on immune cells but may increase glioma cell proliferation. They are attracted by glioma culture supernatant and purified factors including IL-8, VEGF, and TGF- $\beta$  (Birnbaum et al., 2007), but *in vivo* immunosuppressive roles in glioma remain to be determined. Myeloid-derived suppressor cells are another potentially immunosuppressive cell type receiving increasing attention in the context of cancer, and it is noteworthy that they are stimulated by prostaglandin E<sub>2</sub>, which can be secreted by glioma cells (Marx, 2008). Myeloid-derived suppressor cells have also been studied in the context of brain tumor models (Prins et al., 2002), although little is known about their presence at the brain tumor site in patients.

The cellular mediator of immune suppression that is currently the most studied in cancer is the CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cell (T<sub>reg</sub>), which is the best-defined T suppressor cell (Sakaguchi et al., 2008). Either T<sub>regs</sub> are produced as a functionally mature T-cell subset in the thymus (natural T<sub>regs</sub>) or other naïve CD4 T cells can differentiate into induced T<sub>regs</sub> under the influence of certain cytokines including TGF- $\beta$  and IL-2. The role of T<sub>regs</sub> in immune homeostasis is now well defined. They maintain immunological tolerance to self-antigens, and mutations leading to their absence in animals (Scurfy mice) or patients (IPEX), or their inhibition or ablation, leads to autoimmune and inflammatory disease. T<sub>regs</sub> function by an array of cell contact and cytokine-mediated mechanisms to suppress T cells, B cells, NK cells, macrophages, and DCs at both the induction and effector stages of immune responses. In the context of malignancy, T<sub>regs</sub> accumulate in many tumors, including human glioma (El Andaloussi and Lesniak, 2007; Fecci et al., 2006a; Hussain et al., 2006), and T<sub>reg</sub> depletion or inhibition in murine brain tumor models can reveal spontaneous tumor immunity, or enhance induced immunity (Curtin et al., 2008; El Andaloussi et al., 2006; Fecci et al., 2006b; Grauer et al., 2007). While the importance of T<sub>reg</sub> in the antiglioma immune response is widely accepted, there are many challenges in identifying and modulating these cells. Foremost is the lack of a unique marker to identify T<sub>regs</sub>. Most markers are shared with activated effector T cells (e.g., CD25), and the best specific T<sub>reg</sub> marker in mice (the transcription factor Foxp3) is intracellular (and so inaccessible to antibodies *in vivo*), and expression is not restricted to T<sub>reg</sub> in human T cells.

## 48.5 Identification of Glioma-Associated Antigens

Advancing our understanding of the mechanisms underlying the induction of an immune effector response against the tumor, and how it may sometimes be compromised, is only possible if we are able to define the specificity of tumor immunity. This is a major and ongoing challenge for human glioma.

### 48.5.1 *Identifying Tumor-Associated-Antigens* (“Reverse Immunology”)

Significant progress in defining the nature of the antitumor response has been made by the discovery and characterization of TAAs, beginning with the report of the first melanoma antigen (MAGE) in 1991 (Van den Eynde et al., 1995; van der Bruggen et al., 1991). Intensive research is underway into the use of TAAs as potential targets of immune-based cancer treatments. The key TAAs under investigation are MAGE-3, MART-1, tyrosinase, TRP-2, and gp100 for melanoma (Panelli et al., 2000; Parkhurst et al., 1998, 1996; Ribas, 2006; Ribas et al., 2000); PSA and PAP for prostate cancer (Murphy et al., 1996 1999); survivin for many solid tumors (Katoh et al., 2003); and HER-2/neu for breast and ovarian cancers (Disis et al., 2002, 2004; Knutson and Disis, 2001; Knutson et al., 2001). A prominent issue that distinguishes cerebral malignancies from other tumors, such as melanoma, is the paucity of well-defined tumor-specific antigens. Indeed, the difficulties of immune response monitoring are particularly acute in patients with malignant gliomas because, in contrast to patients with melanoma, there are few extensively characterized tumor antigens that can be recognized by T cells. Unless advances in this domain occur, rational advances in immune-based brain tumor therapy may be significantly hampered.

In recent years, genome-wide techniques have dramatically accelerated our ability to obtain molecular profiles of gene defects in gliomas, resulting in a more readily accessible source of information on the genes and gene products that produce antigens (TCGA, 2008). Advances in brain tumor “immunomics” have led to the recent identification of several TAAs expressed by human gliomas. These include tumor-specific antigens unique to the oncogenic transformation process (EGFRvIII) or overexpressed following activation of oncogenic pathways (EphA2, IL-13R $\alpha$ ) (Husain et al., 2001; Kuwashima et al., 2005; Okano et al., 2002; Saikali et al., 2007; Shimato et al., 2008; Wu et al., 2006) (see also Chapter 35), cancer-testes antigens normally expressed only during development (cancer-testes antigens; NY-ESO-1, MAGE, GAGE, SART, SSX) (Bodey et al., 2008; Chi et al., 1997; Sahin et al., 2000), neuroectodermal antigens involved in pigment synthesis (e.g., melanoma-associated antigens; gp100, tyrosinase, TRP-1, TRP-2) (Liu et al., 2004a, b; Saikali et al., 2007; Zhang et al., 2008), and other antigens associated with proliferation and cell survival (hTERT, Survivin, B-cyclin, Her-2/neu) (Katoh et al., 2003; Liu et al.,

2004a; Saikali et al., 2007; Ueda et al., 2007; Zhang et al., 2008). Recent studies have additionally suggested that many human gliomas are latently infected with human cytomegalovirus (CMV) (Cobbs et al., 2002; Mitchell et al., 2008), and that CMV may be a clinically relevant target for immune-based therapies (Prins et al., 2008a).

Many TAAs were originally identified via serological analysis of tumor antigens by recombinant cDNA expression cloning (SEREX) in immunogenic solid tumors such as melanoma (Chen et al., 1997). A similar attempt to identify antigens recognized by the immune system in an IL-4-secreting rat 9L glioma immunization successfully identified mouse Id-associated protein 1 (MIDA) as an antigen that could induce antitumor immunity (Okada et al., 2001). In human gliomas, however, attempts to identify T-cell antigens have been less successful (Pallasch et al., 2005; Schmits et al., 2002). Nevertheless, some recent studies have demonstrated that many established human glioma cell lines express well-characterized TAAs that can be recognized by antigen-specific CD8<sup>+</sup> T cells (Zhang et al., 2007). Interestingly, the pattern of antigen expression seems to differ between adult and pediatric human gliomas (Okada et al., 2008; Zhang et al., 2008).

### ***48.5.2 Microarray Technology and Tumor-Associated-Antigens***

With the rapid advances provided by high throughput microarray chip technology, the entire compendium of genes showing elevated expression in tumors will soon be established, and the repertoire of potential glioma-specific antigens defined (Horvath et al., 2006; Liao et al., 2000; Mischel et al., 2003a, b). Furthermore, recent progress in our understanding of tumor immunology can link the information generated from gene-expression profiling of CNS tumors to the identification of precise immunogenic epitopes that can serve as useful targets for specific immunotherapy (Freije et al., 2004). For instance, since T cells can only recognize antigens in the context of MHC, tumor-associated genes can be scrutinized for amino acid sequences that can interact with relevant MHC molecules and efficiently bind via the proteosomal degradation system. Candidate tumor-specific peptide epitopes for CTLs can now be identified using available computer algorithms that predict the MHC binding affinities of specific peptide and epitope sequences ([http://bimas.dcrt.nih.gov/molbio/hla\\_bind/](http://bimas.dcrt.nih.gov/molbio/hla_bind/), <http://www.uni-tuebingen.de/uni/kxi/>). By subsequently testing the stability of MHC molecules with candidate tumor-specific peptides in vitro, an estimation of the potential immunogenicity of an in silico-identified antigen can be determined (Maecker et al., 2001). The immunogenicity of human cancer genes or their predicted CTL peptide epitopes can also be assessed in animal models using HLA transgenic mice (Butterfield et al., 2001), providing even better estimates for immunotherapy targets in vivo. Recently, experiments involving the screening of human tumor samples for CTL reactivity have

identified human glioma-associated antigen epitopes to EphA2 and IL13R $\alpha$  specifically recognized by the immune system and associated with extended survival (Okano et al., 2002; Ueda et al., 2007). Based on these recent advances, it is suggested that cancer genomics can be directly linked to tumor immunotherapy by “reverse immunology,” which paves the way for the design of new, more specific targets for brain tumor immunotherapy.

## **48.6 Preclinical Studies of T-Cell Immunity to Target Brain Tumors**

### ***48.6.1 Passive Immunotherapy***

#### **48.6.1.1 Adoptive Transfer**

Adoptive transfer of immune cells is the introduction (adoption) into the patient of either autologous or allogeneic immune cells that may, or may not, have been stimulated *in vitro* with tumor antigens (Mitchell et al., 2003). Sometimes, these cells are injected into the tumor cavity to maximize the exposure of the infused cells to tumor cells, while other strategies call for systemic infusion. Cytolytic effector cells are cultured *in vitro* with cytokines to activate them to attack tumor tissue *in vivo*. This approach was not clinically applicable until the discovery of IL-2 and other cytokines that modulate T-cell growth and survival. Before that time, it was not possible to obtain sufficient numbers of immune cells and maintain them in culture during the *in vitro* sensitization period. IL-2, originally called T-cell growth factor, is a cytokine that mediates lymphocyte activation and stimulates division.

In recent years, the ability to adoptively transfer defined tumor antigen-specific T cells has greatly increased our knowledge of critical homing markers, cytokine dependence and *in vivo* trafficking patterns necessary to target and eradicate tumors in the brain. Preclinical studies in rat and murine glioma models first demonstrated that polyclonal, glioma-specific T cells could eradicate CNS gliomas (Baldwin et al., 1997; Kruse et al., 1990; Plautz et al., 1997; Rice et al., 1997) and was associated with enhanced trafficking of these lymphocytes to the brain (Hazelrigg et al., 2002). Subsequent work demonstrated that the draining LN population, from which these lymphocytes are isolated, also includes suppressor populations that can impair the efficacy of the adoptively transferred population (Peng et al., 2002). Removal of the CD62L<sup>high</sup> population enriched the lymphocyte population with enhanced antitumor activity (Plautz et al., 1997). This contrasts with other recent studies on adoptive immunity, in which clonally derived CD8<sup>+</sup> T lymphocytes were used. In this case, the CD62L<sup>+</sup> population identified CD8<sup>+</sup> T cells of the central memory phenotype that possess enhanced trafficking, expansion, and survival (Klebanoff et al., 2005). Different models may account for the differences

observed but suggest that careful characterization of the cellular infiltrate is critical for antitumor activity.

#### **48.6.1.2 Cytokines**

Cytokines can influence the priming of tumor antigen-specific T cells prior to adoptive transfer and subsequently alter their homing patterns, survival, and antitumor activity. In an adoptive transfer model where antigen-specific CD8<sup>+</sup> T cells were polarized to a type 1 (Tc1) cytokine profile using IL-12 and blocking antibodies to IL-4 (Nishimura et al., 2006), the T cells showed preferential homing to intracranial tumors (probably due to high expression of VLA-4/ $\alpha_4\beta_1$  integrin, as discussed above) and enhanced antitumor efficacy (Sasaki et al., 2007). Other work has demonstrated that chemokines, such as tumor-derived monocyte chemoattractant protein-1 (MCP-1) (Desbaillets et al., 1999), can be sufficient to mediate the tropism of tumor-specific T cells to intracranial tumors (Brown et al., 2007). Most recent studies have also demonstrated that tumor-specific CD8<sup>+</sup> T cells primed with antigen, IL-2, and IL-15 can lead to enhanced trafficking to intracranial tumors and migration through the cervical LNs, spleen, and bone marrow (Prins et al., 2008b). In this adoptive transfer setting, endogenous host cytokines are also important for the survival and effector functions of the transferred cells. Host lymphodepletion, via whole body radiation or cytotoxic chemotherapy, can facilitate the homeostatic expansion of adoptively transferred T cells by improved competition for endogenous cytokines (Gattinoni et al., 2005), such as IL-7 and IL-15, eliminating endogenous regulatory T cells (Chang et al., 1986) and/or enhancing the intratumoral accumulation of the transferred cells (Wang et al., 2005).

#### **48.6.1.3 Toll- Like Receptor Agonists**

More recent work has demonstrated that TLR agonists, such as imiquimod (Prins et al., 2006b) and STAT-3 inhibitors (Fujita et al., 2008) can increase serum cytokines and enhance the antitumor activity of adoptively transferred T cells in intracranial tumor-bearing mice. Such studies collectively suggest that the cytokine microenvironment can impose phenotypic changes on tumor-specific T cells that can alter their in vivo trafficking patterns, function, and tropism for tumors growing in anatomically distinct compartments.

### **48.6.2 Active Immunotherapy (Tumor Vaccines)**

Active immunotherapy strategies (tumor vaccines) require administration of the antigenic material to induce an (primary) immune response, in effect, a vaccination. Most tumor antigens are poor immunogens, and thus active immunotherapy usually includes the use of an “adjuvant” (e.g., *Bacille*

*Calmette-Guerin* or cytokines) that enhances the immune response by prolonging the time of exposure to antigen and by increasing the activity of APCs. DC-based therapies are one type of active immunotherapy (Liau et al., 1999). Other approaches have included irradiated whole tumor cell vaccines and cytokine immuno-gene therapy strategies (Glick et al., 2001; Okada et al., 2003).

#### 48.6.2.1 Dendritic Cell-Based Vaccines

As discussed earlier, DCs are the most potent APCs in the body, due in part to their high expression of MHC class I and II, and costimulatory molecules, and their secretion of cytokines that promote T cell priming (e.g., IL-12). Immune activation by DCs facilitates engagement of all effector mechanisms of the immune system, such as CTLs and CD4<sup>+</sup> helper T cells (T<sub>h</sub>), as well as NK cells and antibodies. The use of DCs for cancer immunotherapy became feasible a decade ago with the advent of new techniques to grow these cells from bone marrow precursors in animals (Thurner et al., 1999) and from CD14<sup>+</sup> monocytes in human peripheral blood (Kiertcher and Roth, 1996). These advantages prompted a wide array of preclinical studies to define the constraints and mechanisms by which DC can prime tumor-specific T-cell responses.

DC-based immunotherapy can accommodate many different forms of antigen, but chief among them is the use of materials derived from autologous tumor cells. DC pulsed with tumor-eluted peptides (Liau et al., 1999), apoptotic/necrotic tumor cells (Siesjo et al., 1996), tumor lysates (Aoki et al., 2001; Ni et al., 2001; Pellegatta et al., 2006), tumor-derived RNA (Insug et al., 2002), and even glioma neurospheres (Pellegatta et al., 2006) can induce relevant antitumor immunity for tumors in the brain. The advantage of using such materials is that this approach is applicable against neoplasms for which immunogenic tumor-specific or tumor-associated antigens are unknown. The disadvantages, however, are that such autologous tumor extracts may be heterogeneous and are therefore difficult to characterize and quantitate. Also, the use of unfractionated tumor material may lead to relatively low concentrations of effective immunogens in the mixture, as antigenic tumor peptides may conceivably be diluted by relatively non-immunogenic proteins and thereby lower the immunogenicity of the vaccine. Furthermore, a risk for inducing immunity against self-antigens that may lead to autoimmune encephalitis is real (Bigner et al., 1981).

Alternative forms of antigen loading for DC-based vaccines include the use of defined peptides and genetic transfection (Morse et al., 2005; Ribas, 2006). As such, it was demonstrated that tumor-specific, MHC class I-restricted peptides can be pulsed onto DCs and used to induce significant antitumor immunity to intracranial tumors (Ciesielski et al., 2008; Hatano et al., 2004; Heimberger et al., 2002, 2003; Prins et al., 2003). Similarly, viral transduction of DCs with tumor antigens (Broder et al., 2003), cytokines (Kim et al., 2006; Tsugawa et al., 2004; Yamanaka et al., 2003b), and/or fusions of DCs and tumor cells (Kjaergaard et al., 2005) has generated antitumor immunity that is being



translated into clinical trials. Currently, DCs are recognized as a promising vehicle for active immunotherapy of cancer. Numerous animal experiments have demonstrated the potential of DC-based immunotherapy in both protecting mice from tumor formation and eliminating established tumors. However, other work has shown that DC vaccination is more effective at priming tumor-specific T-cell responses but relatively inefficient for boosting the same response (Jouanneau et al., 2006). Thus, there is a rationale for incorporating DC vaccination with chemotherapy, adjuvants, and/or viral vectors that may synergize to induce therapeutic antitumor immunity.

#### 48.6.2.2 Adjuvants

It is becoming clear from preclinical studies and human trials that enhancement of DC vaccines with adjuvants will be required to generate an antitumor immune response that is both reproducibly effective and long lasting. DCs express numerous TLRs (see above) that are important in host defense against bacteria and other microbial pathogens (Barrat and Coffman, 2008; Barton and Medzhitov, 2002; Pulendran, 2005). Stimulation of TLRs ultimately results in gene expression profiles that lead to the production of cytokines including TNF- $\alpha$ , IL-1, IL-6, and IL-12 as well as type I and type II IFNs (IFN- $\alpha$  and IFN- $\gamma$ ) (Barton and Medzhitov, 2002; Pulendran, 2005). TLR ligation on DC induces a maturational signal that can up-regulate MHC and costimulatory molecule expression, induce migration to LNs, and induce expression of cytokines that can promote and enhance T-cell priming against tumors (Steinman and Banchereau, 2007). Since it is commonly believed that activated DCs can prime T-cell responses more effectively (Steinman and Banchereau, 2007), numerous groups have utilized adjuvants to effectively mature DCs for cancer immunotherapy. For example, the TLR-7 agonist, imiquimod (Aldara<sup>®</sup>), could synergize with peptide-pulsed DC vaccination paradigms to induce effective antitumor immunity to intracranial tumors (Prins et al., 2006b). Imiquimod enhanced not only DC survival and trafficking to LNs but also the priming of self, tumor antigen-specific T cells (Prins et al., 2006b). Similar findings were reported with poly-ICLC, a TLR-3 agonist (Zhu et al., 2007), and CpG, a TLR-9 agonist (Wu et al., 2007).

#### 48.6.2.3 Blocking Regulatory T Cells (T<sub>regs</sub>)

The blockade of immuno-inhibitory molecules can also generate antitumor immunity and/or synergize with active vaccination approaches for the treatment of CNS tumors. T<sub>regs</sub> (see above) have recently been shown to accumulate in the peripheral blood and tumors of mice (Grauer et al., 2007) and patients (El Andaloussi and Lesniak, 2007; Fecci et al., 2006a) with malignant glioma, and this was associated with the degree of malignancy and immune competency (El Andaloussi and Lesniak, 2007; Fecci et al., 2006a; Heimberger et al., 2008; Learn et al., 2006). These data suggest that a tumor-derived factor may

influence this type of immune evasion so as to prevent proper immune surveillance of tumors arising in the CNS. As such, recent studies have demonstrated that blockade of the negative costimulatory molecule, CTLA-4, can ameliorate changes to the CD4<sup>+</sup> T-cell compartment and confer long-term survival in intracranial tumor-bearing animals (Fecci et al., 2007). Similarly, others have recently demonstrated that functional blockade of T<sub>regs</sub> with in vivo CD25 mAb administration can enhance the antitumor immunity to vaccines in a prophylactic setting (Fecci et al., 2006b; Grauer et al., 2008). However, these studies highlight the difficulties of T<sub>reg</sub> depletion, since the CD25 mAb (PC61) used therein can also bind and inhibit activated T cells, completely removing any beneficial effect of an active vaccination immunotherapy in established intracranial tumor-bearing mice (Curtin et al., 2008). Such studies highlight the importance of the T-cell compartment in CNS tumor immunity. New methodologies are being explored to functionally reverse the T<sub>reg</sub> influence in combination with antitumor vaccination strategies.

#### 48.6.2.4 Immune Gene Therapy

Other studies of cancer vaccines have used irradiated whole tumor cells modified with cytokine genes (reviewed in (Okada and Pollack, 2004)), viral vectors (reviewed in (Curtin et al., 2005)), or even live, attenuated bacterial vectors (Liau et al., 2002; Prins et al., 2006a) to generate antitumor immunity. The pioneering cytokine gene therapy studies performed over a decade ago demonstrated that cytokine-secreting tumor cells could induce antitumor immunity. With CNS tumor models, cytokine-gene therapy studies have been able to demonstrate efficacious antitumor immunity to intracranial gliomas by induction of IL-2, IL-4, IL-6, IL-7, IL-12, GM-CSF, mM-CSF, and IFN- $\alpha/\beta/\gamma$  (Glick et al., 2001, Dey et al., 2006). Such cytokine-secreting tumors revealed that multiple facets of the cellular immune response could be mobilized to induce antitumor immunity. Other groups have utilized intracranial injections of viral vectors, which encode cytokines and/or conditional cytotoxicity (HSV-TK) (Ali et al., 2005; Curtin et al., 2005) (see also Chapters 46 and 47).

#### 48.6.2.5 Bacterial/Viral-Based Vaccines

Live bacteria and/or viruses may also serve as the basis for active immunotherapies against brain tumors (Chabalgoity et al., 2002). Viral/bacterial infections and the resulting tissue damage can also provide the appropriate “danger signals” (Matzinger, 1994) to attract professional APC necessary for adequate antigen presentation. CTL-mediated immunity can be induced using live, attenuated bacterial or viral vectors that both stimulate the innate immune system and simultaneously deliver antigens (Chabalgoity et al., 2002).

For instance, *Listeria monocytogenes* (*LM*) is a facultative, gram-positive intracellular bacterium that is able to enter host cells, escape from the endocytic vesicle, multiply within the cytoplasm, and spread directly from cell to cell

without encountering the extracellular milieu. Antigens expressed by *LM* can access both MHC class I and class II processing pathways and are presented to both CD8<sup>+</sup> and CD4<sup>+</sup> T cells. Additionally, *LM* has been shown to stimulate TLRs (Barton and Medzhitov, 2002) on the surface of APC and activate internal pattern recognition molecules, which may contribute to its immuno-stimulatory action (Ulevitch, 2004). In published studies, it has been demonstrated that immunization with an attenuated, recombinant *Listeria monocytogenes* (*rLM*) expressing the lymphocytic choriomeningitis virus nucleoprotein (LCMV-NP) led to the rejection of a glioma expressing the heterologous NP antigen (Liau et al., 2002). Interestingly, animals clearing these tumors were subsequently immune to re-challenge with subcutaneous and intracranial glioma cells that did not express NP, suggesting that epitope spreading had occurred, which is a phenomenon whereby T cells can recognize other endogenous glioma epitopes along with the targeted antigen(s) (Liau et al., 2002). Given the heterogeneity of human brain tumors and the possibility for the immune escape, exploiting the process of epitope spreading will be valuable in the clinical context of future tumor vaccine design. These studies reveal the complexity of the multiple mechanisms by which antitumor immunity can be generated.

## **48.7 Clinical Trials of Cellular Immunotherapy for Brain Tumors**

### ***48.7.1 Lymphokine-Activated Killer Cells***

The majority of previous clinical adoptive immunotherapy trials for brain tumors have employed lymphokine-activated killer (LAK) or mitogen-activated killer cells. LAK cells are peripheral blood lymphocytes functionally defined by their ability to lyse NK-resistant tumor targets *in vitro* following stimulation with IL-2 and/or mitogens. LAK cells have been implanted into the resection cavity during surgery (Dillman et al., 2004; Hayes et al., 1995; Merchant et al., 1988a, b), and sporadic clinical responses were observed. However, LAK cell therapy is nonspecific in that the cells are not stimulated *in vitro* with any glioma-specific antigens. The limitations of LAK cell adoptive transfer to eradicate tumor cells in clinical trials may be due to its inherent non-specificity, the fact that cells do not migrate efficiently to tumor sites, and/or the induction of diffuse cerebral edema from IL-2 and other cytokines.

### ***48.7.2 Cytotoxic T Lymphocytes***

One refinement to the previous adoptive transfer approaches is to stimulate lymphocytes *in vivo* with autologous tumor cells to generate MHC class I-restricted CTLs that are then expanded *in vitro* and re-infused into patients

(Kruse et al., 1997; Merchant et al., 1997). The theory is that antigen-stimulated CTLs would be more specific than LAK cells. Clinical trials using adoptive transfer of activated CTLs have produced somewhat encouraging initial results (Kruse et al., 1997; Plautz et al., 2000; Wood et al., 2000). However, because these pilot phase I trials were designed primarily to demonstrate feasibility and safety, definitive evaluation of efficacy will require further study.

Often, pilot trials of adoptive immunotherapy have suggested efficacy (Ishikawa et al., 2004; Quattrocchi et al., 1999), but they have not been followed up with phase II or III larger trials designed to show true improvement in survival. Adoptive transfer studies in melanoma have been particularly encouraging, and currently ~50% of treated patients will have an objective clinical response by RECIST criteria (Dudley et al., 2005). However, the ability to clone and expand high-affinity tumor-infiltrating lymphocytes is currently restricted to a few centers.

A newer option that may become feasible in the future, when glioma-specific TCRs are cloned, will be to use genetic engineering to specifically confer tumor recognition to normal lymphocytes (Morgan et al., 2006). Alternatively, the use of bispecific antibodies, which have the potential to engage all CTLs in patients for lysis of cancer cells, may have therapeutic potential for the treatment of malignant diseases. Contrary to other antibody-based approaches, which call for injection of massive doses of purified antibodies, a recent study has shown tumor regression in non-Hodgkin's lymphoma patients by treating patients with very low doses of recombinant bispecific antibodies (blinatumomab), which render the cancer cells sensitive to almost all CTLs that they may encounter (Bargou et al., 2008). Conceivably, such bispecific single-chain antibodies, where one variable region recognizes an antigen on tumor cells and the other variable region binds to CD3, may be engineered for solid tumors, such as malignant gliomas.

Overall, it appears that adoptive immunotherapy may offer certain advantages that active vaccination strategies cannot provide: (1) T cells are expanded in the absence of tumor-derived soluble factors and (2) CTLs can be grown to extremely large numbers sufficient for infusion (Wang et al., 2004). A summary of recently published clinical trials of adoptive cellular immunotherapy for malignant gliomas is presented in Table 48.2.

### ***48.7.3 Dendritic Cell Vaccination Trials***

Approaches that combine adoptive transfer of antigen-pulsed DCs for tumor vaccination in malignant glioma patients are currently under active investigation at several different centers around the world (Caruso et al., 2004; De Vleeschouwer et al., 2008; Kikuchi et al., 2004, 2001; Liau et al., 2005; Yamanaka et al., 2003a, 2005; Yu et al., 2004; Yu et al., 2001). This strategy involves ex vivo exposure of a patient's DCs to their tumor antigen followed by their in vivo infusion to stimulate an endogenous immune response (Liau et al., 1999; Prins et al., 2003). The theory behind this therapeutic strategy is based

**Table 48.2** Summary of clinical trials of *adoptive* cellular immunotherapy for malignant gliomas

Therapy	Phase	Tumor HISTOLOGY	Responses	Survival	References
Allo-CTL + IL-2	Phase I (n = 5)	GBM (2); AA (1); AO (2) [recurrent]	NR	AA/AO patients with SD > 28 months	Kruse et al. (1997)
TILs + IL-2	Phase I (n = 6)	GBM; AA [recurrent]	1 CR; 2 PR	NR	Quattrocchi et al. (1999)
Activated CTL from lymph nodes	Phase I (n = 12)	GBM (6); AA(4); LGA (2) [newly diagnosed]	4 PR	3 patients > 2 yr	Plautz et al. (2000)
Activated CTL from PBMC	Phase I (n = 9)	GBM; AA [recurrent]	3 PR	2/9 patients > 4 yr	Wood et al. (2000)
LAK cells + IL-2	Phase I (n = 28)	GBM; AA [recurrent]	Lymphocytic infiltration; locally ↑ IL-2 & IFN-γ	6/28 patients > 2 yr	Hayes et al. (2001)
NK cells	Phase I (n = 9)	GBM (3); AA (6) [recurrent]	3 PR; 2 MR	NR	Ishikawa et al. (2004)
LAK cells	Phase I (n = 40)	GBM [recurrent]	NR	34% 1-yr survival; Median OS = 17.5 months	Dillman et al. (2004)

TIL = tumor-infiltrating lymphocyte; GBM = glioblastoma multiforme; AA = anaplastic astrocytoma; AO = anaplastic oligodendroglioma; LGA = low-grade (grade II) astrocytoma; PFS = progression-free survival; CR = complete response; PR = partial response; MR = minimal response; OS = overall survival; NR = not reported.

upon evidence that tumor cells are poor APCs, and do not adequately stimulate endogenous professional APCs. The lack of immune activation may affect not only CD8<sup>+</sup> T cells but also the CD4<sup>+</sup> “helper” T cells (T<sub>h</sub>) that require antigen presentation in association with MHC class II. Unless tumor antigens are secreted as soluble proteins and can be processed by professional APCs, the CTL response will be handicapped by the lack of critical T<sub>h</sub> function such as cytokine secretion and full DC activation. Soluble antigen may be released by tumor cells in later stages with the development of necrotic areas; but by this time, the tumor may be past the threshold from which its growth can be impeded by immune activity. To circumvent this, investigators are using *ex vivo* cytokine stimulation of DC and antigen exposure of autologous DCs with autologous tumor lysate, tumor peptides, or tumor cell fusions (Table 48.3).

**Table 48.3** Summary of clinical trials of *active immunotherapy (tumor vaccines)* for adult malignant gliomas

Therapy	Phase	Tumor histology	Responses	Survival	References
Tumor cells modified with Newcastle disease virus (NDV)	Phase I ( <i>n</i> = 11)	GBM [newly diagnosed]	Local skin reaction	Median OS = 46 wks	Schneider et al. (2001)
DC pulsed with acid-eluted tumor peptides	Phase I ( <i>n</i> = 9)	GBM (7); AA (2) [recurrent]	2/4 patients with ↑ lymphocytic infiltration		Yu et al. (2001)
DC-glioma cell fusions	Phase I ( <i>n</i> = 8)	GBM; AA	4/5 patients with ↑ CD16+ & CD56+ cells; ↑ IFN-γ in peripheral blood	NR	Kikuchi et al. (2001)
Tumor cell + IL4-transfected fibroblasts	Phase I ( <i>n</i> = 1)	GBM	Local CD4+, CD8+, and CD1a+ T cells ↑ with IL-4 produced at injection site	Patient survived 10 months	Okada et al. (2003)
DC pulsed with tumor lysate (intradermal + intratumoral via Ommaya reservoir)	Phase I/II ( <i>n</i> = 10)	GBM (7); AA (3)	2/5 patients with ↑ ELISPOT activity; 3/10 with ↑ DTH; 2 with ↑ lymphocytic infiltration	NR	Yamanaka et al. (2003a)
DC pulsed with tumor lysate	Phase I ( <i>n</i> = 14)	GBM (8); AA (6) [recurrent]	6/10 with ↑ IFN-γ in peripheral blood; 3/6 with ↑ lymphocytic infiltration	Median OS = 133 wks	Yu et al. (2004)
DC-glioma cell fusion + IL-12	Phase I ( <i>n</i> = 15)	GBM; AA	4/15 with >50% ↓ tumor size	NR	Kikuchi et al. (2004)
Tumor cell modified with Newcastle disease virus (NVD)	Phase II ( <i>n</i> = 23)	GBM	↑ DTH; ↑ tumor-infiltrating lymphocytes	Median OS = 100 weeks (vs. 49 weeks in controls, <i>n</i> = 87); 39% 2-yr survival rate	Steiner et al. (2004)
DC pulsed with acid-eluted tumor peptides	Phase I ( <i>n</i> = 12)	GBM (12) [7 newly diagnosed; 5 recurrent]	6/12 with ↑ CTL activity; 4/8 ↑ tumor-infiltrating lymphocytes	Median OS = 23.4 months; 50% 2-yr survival rate	Liau et al. (2005)

Table 48.3 (continued)

Therapy	Phase	Tumor histology	Responses	Survival	References
DC pulsed with tumor lysate	Phase II ( $n=32$ )	GBM [11 newly diagnosed, 23 recurrent]	14/26 with > 1.5-fold ↑ in IFN- $\gamma$ levels	Mean OS = 21.4 months; 41% 2-yr survival rate for vaccine responders	Wheeler et al. (2008)
DC pulsed with tumor lysate	Phase I/II ( $n=56$ )	GBM [recurrent]	9/17 with pos. DTH post-vaccination	Median OS = 9.6 months; 14.8% 2-yr survival rate	De Vleeschouwer et al. (2008)

GBM = glioblastoma multiforme; AA = anaplastic astrocytoma; DTH = delayed-type hypersensitivity; PR = partial response; OS = overall survival; NR = not reported.

In a recent phase I clinical trial with 5-year follow-up, 12 patients with newly diagnosed ( $n = 7$ ) or recurrent ( $n = 5$ ) glioblastoma were enrolled. Patients received standard of care, which included surgery followed by external beam radiation therapy. Glioma cells were dissociated from the tumor specimen and cultured in vitro. After approximately 5 weeks in culture, MHC-bound tumor peptides were eluted from the surface of the tumor cells using an acid-elution protocol (Liau et al., 1999). The patient's own autologous DCs were then harvested from peripheral blood mononuclear cells and subsequently loaded with the autologous acid-eluted tumor peptides. The patients then received three intradermal injections of peptide-pulsed DCs at 2-week intervals. Although this phase I study was not powered to detect clinical efficacy, it provided further evidence on the feasibility, safety, and in vivo bioactivity of autologous peptide-pulsed DCs in patients with glioblastoma, as did other similar studies (Kikuchi et al., 2004, 2001; Liau et al., 2005; Yajima et al., 2005; Yamanaka et al., 2003a; Yu et al., 2004, 2001). Although admittedly a select population of patients, prolonged survival times and significant immunological responses were observed in some of these patients, which supports the possibility of an immune-related effect on tumor control. A recent study correlated quantitative T-cell immune responses, as measured by IFN- $\gamma$  enhancement, with clinical time to tumor progression (TTP) and survival (Wheeler et al., 2008). Proof of clinical benefit from DC-based vaccines remains to be established in future multi-center phase II clinical trials for malignant glioma patients, which are currently underway.

The following conclusions can be drawn from these DC-based clinical trial data to date. First, DC therapy appears to be relatively safe and well tolerated. For the most part, no serious side effects have been observed with any of these trials. Second, many of these trials report induction of cellular immunity, humoral immunity, or both, against vaccine components. Most importantly, some of these trials report complete/partial response rates or prolonged survival, which suggest that DC-based immunotherapy can significantly impact disease-free and overall survival. The true benefit of these therapies still needs to be assessed in rigorously controlled randomized trials, some of which are underway.

#### ***48.7.4 Bacterial and Viral Tumor Vaccine Trials for Malignant Glioma***

*Salmonella typhimurium* is a gram-negative enterobacterium that causes typhoid fever. These are motile bacteria that have an anaerobic metabolism and, therefore, will thrive in hypoxic environments such as those occurring in tumors. *Salmonella typhimurium*, attenuated by genetically modifying the *purI* and *msbB* genes responsible for virulence and innate immune recognition, respectively, were found to specifically target and localize to transplantable



murine tumors and partially inhibit tumor growth in vivo (Clairmont et al., 2000; Rosenberg et al., 2002). These preclinical results led to a phase I clinical trial of the intravenous administration of attenuated *Salmonella typhimurium* (VNP20009) to patients with metastatic melanoma (Rosenberg et al., 2002). This clinical study showed that the VNP20009 strain of *Salmonella typhimurium* could be safely administered to patients and that some tumor colonization by *Salmonella* was observed at the highest tolerated dose. However, no antitumor effects were seen (Toso et al., 2002).

Viral vaccines have also been investigated for the treatment of malignant gliomas (Schneider et al., 2001). More recently, a Newcastle disease virus vaccine (MTH-68/H) was used in four patients with advanced high-grade glioma, with purported survival times of 5–9 years (Csatory et al., 2004). In a similar, expanded study, a vaccine prepared from patients' tumor cell cultures infected with Newcastle disease virus was used and followed by gamma-irradiation. This was a non-randomized study of 23 vaccinated patients compared with 87 non-vaccinated controls. The 2-year survival rate in the vaccinated patients was 39% as compared with 11% for the controls ( $p < 0.001$ ). This viral-based vaccine appeared to be feasible and safe, and the improved prognosis of the vaccinated patients was substantiated by observed antitumor immune responses (Steiner et al., 2004).

Each of the immune-based therapies outlined above utilizes principles of basic immunology to find a strategy that hopefully brings us closer to the goal of killing the residual microscopic tumor cells that lead to inevitable recurrence of malignant gliomas. Major improvements in our understanding of glioma molecular biology and tumor immunology are now being translated into innovative clinical trials that provide new hope for patients with this devastating disease (see Tables 48.2 and 48.3).

## 48.8 Conclusion

There is challenging work being done today to take basic immunology into the clinical realm. To date, clinical trials of immunotherapy for CNS gliomas have not yet demonstrated objective proof of clinical efficacy in rigorous multi-center phase II and III studies. Nevertheless, such trials should be pursued because of encouraging results in many phase I studies (Salgaller and Liao, 2006). As future testing in this field continues, our ability to design effective, targeted immune therapies will mature and hopefully yield increased therapeutic success.

With this in mind, the priority areas of research and scientific investigation that appear to be most critical at this time involve (1) developing techniques of new antigen identification, based on readily available sources of information on the genes and gene products that are abnormally expressed by primary tumor cells (Ryu et al., 2006) and by examining which of them produce immunogenic

antigens (Andersen et al., 2001); (2) characterizing both CNS and systemic immune responses in patients with brain tumors; (3) developing immunotherapies that are optimized for the particularities of the brain (efficient homing of effector cells and acceptable levels of local inflammation); and (4) considering the problems and challenges posed by patient and tumor heterogeneity. It should be noted that as with any other targeted treatment modality for brain tumors, immunotherapy trials might only realize significant potential clinical efficacy if given to the appropriate subgroup of patients and/or if administered in combination with other therapies. With the current experience in cancer treatments, it appears that simultaneously targeting several components essential to the neoplastic process should provide maximal chances of tumor control. Therefore, therapies based on immuno-enhancement and cancer vaccines could be combined with the traditional surgery, radiation, and chemotherapies, along with molecularly targeted biological agents. Such integrated treatment strategies may prove to be of low toxicity and should be synergistic. In addition, the combined use of conventional treatments within the context of clinical trials of immunotherapy will allow evaluation of efficacy, yet retain the ethical requirements for human investigation.

Over the next decade, the concept of stimulating a patient's natural immunity to produce antitumor responses may lead to the approach of "customized immunotherapy" (Salgaller, 2000) for patients with malignant glioma. Such "personalized immunotherapeutics" may be a potential solution to deal with the important observations of patient and tumor heterogeneity. However, there are still significant obstacles for developing highly patient-selective treatments – the patient accrual on clinical trials is slower, the potential market for an approved product is lower, and so development times to clinical applications are prolonged.

Furthermore, there are significant manufacturing challenges that face the clinical development of many immunotherapeutics, especially with regard to patient-specific vaccines. This is arguably the greatest technical (and financial) hurdle to getting these types of treatments into large-scale, pivotal trials. Because so-called GMP-level facilities on many academic campuses do not meet the stricter regulations of the FDA for non-pilot studies, further clinical development of cellular therapies and biologic agents for brain tumors will require partnerships between international academic medical centers, government agencies, and biotechnology companies in order to overcome the manufacturing challenges that currently impede large-scale, multi-center phase III clinical trials.

As we have pointed out above, there is already a plethora of small-scale phase I/II brain tumor immunotherapy trials that still await confirmation of clinical efficacy. In order to obtain meaningful biological and clinical data from more complicated trials of customized immunotherapy in selected patient groups, larger multi-institutional studies need to be performed with more structured collaborations from academic and community medical centers, the biotechnology industry, and patient advocacy groups.

Currently, immunotherapy is cautiously and deliberately making its way to the patient bedside, as adjuncts to standard modalities of surgery, radiotherapy, and chemotherapy. While the number of clinical trials evaluating such immunotherapeutic strategies is still limited, current advances in the high-throughput production of clinical-grade cellular/biologic therapeutics and molecular/genetic target identification will hopefully spur future clinical development.

## Abbreviations

APC	antigen presenting cell
BBB	blood–brain barrier
CTL	cytotoxic T lymphocyte
DC	dendritic cell
EAE	experimental autoimmune encephalomyelitis
IFN	interferon
LAK	lymphokine activated killer (cell)
LN	lymph node
MHC	major histocompatibility complex
MS	multiple sclerosis
NK	natural killer (cell)
TAA	tumor-associated antigen
TCR	T-cell receptor (for antigen)
TGF	transforming growth factor
TLR	toll-like receptors

**Acknowledgements** We thank Dr Nhu Nam Tran Thang for help in preparing the figures.

## Glossary of Key Immunological Terms

**Autologous** The cells, tissues, and molecules derived from self, as opposed to another individual (cf. syngeneic, allogeneic).

**Adaptive Immunity** Immunity mediated by antigen-specific T and B lymphocytes, so called because it adapts to become more efficient upon repeated exposure to antigens (cf. innate immunity), and can provide immunological memory once the initial source of antigen has been eliminated.

**Adoptive Transfer** The introduction into the patient or animal of biological material (usually cells) of autologous, syngeneic, or allogeneic origin. For tumor immunotherapy, adoptive transfer offers the possibility of administering cells enriched for specific cell types (e.g., CTLs) or specificities (e.g., for tumor-specific or tumor-associated antigens) by appropriate *in vitro* stimulation and expansion.

**T-cell activation** Resting T cells do not proliferate and exert their immunological functions (cytotoxicity, cytokine release) until their TCRs engage their cognate ligand (antigenic peptide associated with an MHC molecule). The

initial antigen-specific activation of mature T cells is referred to as priming and requires particularly stringent conditions to occur. These are generally only achieved by professional APCs (e.g., DCs) which express costimulatory molecules in addition to MHC-peptide complexes. The optimal and most clearly understood conditions for T cell priming are in secondary lymphoid tissues. The conditions for subsequent activation of a T cell that has already been primed (i.e., in a secondary immune response, sometimes referred to as re-activation or re-stimulation) are less demanding and can be achieved by various cell types (including tumor cells) that express the appropriate MHC-peptide complex, without costimulation, and in nonlymphoid tissues including the CNS.

**Allogeneic** The cells, tissues, and histocompatibility molecules derived from a genetically different individual (cf. syngeneic, allogeneic).

**Anergy** A T cell (or a B cell) that is unable to respond to its specific antigen. One way in which this may occur is when antigen is initially presented in the absence of costimulatory molecules; this can limit autoimmune reactions but may also limit antitumor reactions.

**Antigens** Material that can be recognized by the T and B lymphocytes of the adaptive immune system via their antigen receptors. The portion of the material that bound by the antigen receptors is the epitope (see also tumor-specific and tumor-associated antigens)

**Antigen processing and presentation** Antigen processing and presentation are divided into three major categories. In the first, exogenous antigens in the extracellular milieu such as cellular debris and opsonised microbes are taken up by antigen presenting cells (APCs), then degraded within acidic endosomes to generate short peptides, certain of which bind to MHC class II molecules and reach the cell surface to be recognized by CD4 T cells. In the second major route, endogenous antigens (i.e., normal, viral, or aberrant proteins synthesized within the cell) are degraded by the proteasome within the cytosol then transported into the endoplasmic reticulum wherein peptide binding to MHC class I molecules can occur, followed by transport to the cell surface for recognition by CD8 T cells. A third category of antigen presentation called cross-presentation is important in tumor immunology and immunotherapy. Cross-presentation is the pathway in which exogenous antigen is taken up by professional APCs, then processed (degraded into short peptides) and presented at the cell surface bound to MHC class I molecules. The most important cell responsible for cross-presentation is the DC. Cross-presentation is particularly important when malignant transformation occurs in parenchymal cells, rather than in professional APCs. For CD8 T cells to be alerted to such threats requires the intervention of an adjacent professional APC such as a DC, which can acquire material derived from the transformed cell (e.g., from dying cells or cellular debris) and cross-present this exogenous antigen to activate naïve CD8 T cells. Tumor vaccines used in immunotherapy need to exploit the cross-presentation pathway in order to activate tumor-specific CD8 T cells.

**Antigen Presenting Cells (APCs)** Cells capable of presenting peptide antigens bound to MHC molecules to T cells. The so-called professional APCs (DCs, macrophages, B cells) also express costimulatory molecules and are capable of activating naïve T cells. The most potent professional APCs are the DCs which (unlike the other professional APCs) have no other major biological function.

**Autoimmunity** A T- or B-cell mediated response directed against antigens of the individual's own body.

**B cells or B-lymphocytes** Lymphocytes of the adaptive immune system that develop in the bone marrow, which express clonally distributed antigen receptors, and which are stimulated by antigen in secondary lymphoid organs to differentiate into plasma cells that secrete antibodies.

**Costimulatory molecules and signals** For activation of naïve T cells, they must receive a first signal from MHC-peptide antigen, and a second costimulatory signal delivered by costimulatory molecules on the APC. CD80 and CD86 are major costimulatory molecules on APC, which bind to CD28 on T cells, which then transduces a costimulatory signal to the cell.

**Cytotoxic T lymphocytes (CTLs)** Fully activated CD8 T cells able to kill target cells expressing their cognate MHC-peptide antigen. A major cytotoxic mechanism (shared by NK cells) is the polarized release of the contents of lytic granules stored within the effector cell directly onto a target cell (e.g., a tumor cell). The principle cytotoxic molecules are perforin, granzymes, and granulysin, and they function by inducing apoptosis in the target cell. Other cytotoxic mechanisms can include cell surface molecules such as Fas ligand, and cytokines such as tumor necrosis factor.

**Dendritic Cells (DCs)** The most efficient professional APCs, of bone marrow origin, which have a branched, dendritic morphology. They fulfill different functions according to their differentiation state. In nonlymphoid tissues, DCs are efficient at phagocytosing and processing antigens and are termed immature. Upon activation via receptors such as TLRs, DCs become mature and migrate to secondary lymphoid tissue, they express high levels of costimulatory and MHC molecules, and they efficiently activate naïve T cells.

**Epitope** The part of an antigen that is bound by an antibody molecule (a B-cell epitope), or that can bind to an MHC molecule and stimulate T cells (a T-cell epitope).

**HLA transgenic mice** Transgenic mice that express a human histocompatibility molecule. These models allow *in vivo* studies of mouse T-cell immune responses specific for antigens defined in humans of the same HLA-type.

**Immunological Synapse** The immunological synapse is the stable interface between a T cell and target cell presenting cognate MHC peptide. It forms through cell-cell interaction of adhesion and signaling molecules. Polarized release of cytokines or cytotoxic molecules into the space formed at the synapse can ensure antigen specificity of T-cell effector function even when mediated by antigen non-specific molecules.

**Innate Immunity** Host immune defenses that react rapidly to infection or danger by means of germline-encoded pattern or pathogen recognition receptors. In contrast to adaptive immune responses, innate immune responses do not become more efficient upon repeat exposure to the original stimulus.

**Lymphocytes** A class of mononuclear leukocytes capable of migrating in blood, lymph, lymphoid, and nonlymphoid tissues according to their state of activation or differentiation. The small lymphocytes of the blood encompass the B and T cells of the adaptive immune system, which bear clonally distributed antigen receptors. Mature lymphocytes are termed naïve before they are stimulated by antigen. Large granular lymphocytes are the NK cells of the innate immune system.

**Lymphoid Organs** Primary lymphoid organs are the organized tissues where lymphocytes are formed, i.e., the bone marrow (for B cells) and the thymus (for T cells). The secondary lymphoid organs are the main site of encounter between APC and naïve T and B lymphocytes, and subsequent lymphocyte activation. The principal secondary lymphoid tissues are the spleen, lymph nodes (LNs), and the mucosa-associated lymphoid tissues (such as the tonsils and Peyer's patches).

**Major Histocompatibility Complex (MHC)** A cluster of genes encoding MHC molecules, a family of polymorphic cell surface glycoproteins called HLA molecules in humans, which are responsible for presenting peptide antigens to T cells. MHC class I molecules present peptides to CD8 T cells and are constitutively expressed on most cells of the body (but notably, are absent or at low levels on astrocytes, oligodendrocytes, and neurons). MHC class II molecules are mainly expressed on professional APCs and present peptide antigens to CD4 T cells.

**Macrophages and microglial cells** Macrophages are a family of large mononuclear phagocytes that can exist in many forms in different tissues and pathologies. They derive from blood monocytes which are in turn derived from the bone marrow. They can function as APCs (although they do not migrate to lymphoid tissue like DCs), they can exert effector functions against pathogens and tumors, but they can also promote tumor invasion and angiogenesis. Macrophages associated with the CNS can be distinguished according to the compartment in which they are found. Macrophages in the perivascular space, the leptomeninges, and the choroid plexus are phenotypically very similar to other tissue macrophages. Macrophages of the brain parenchyma on the other hand express only low levels of most classical macrophage markers under non-inflammatory conditions and have a ramified morphology. These cells are referred to as microglial cells, and upon activation they can differentiate to acquire macrophage phenotype and function and may even express the DC marker CD11c under some conditions.

**Natural Killer (NK) Cells** These cells are large, granular, cytotoxic lymphocytes, but unlike the T and B lymphocytes, they mediate innate immune responses. Their most important functions are cytotoxicity and IFN- $\gamma$

secretion, which are regulated by the balance of signals transduced through activating and inhibitory receptors expressed at the NK cell surface.

**Pathogen/Pattern Recognition Receptors** These receptors are germline encoded and detect conserved patterns present on pathogens, as well as material from stressed or dying cells. Cells of the innate immune system always express some of these receptors, but they are present on other cell types as well. They include the toll-like receptors (TLRs).

**Serological Identification of Antigens by Expression Cloning (SEREX)** A technique by which patient antibodies from serum can be used to screen tumor cell proteins for those which elicit immune responses. A cDNA expression library is initially made of a tumor. This library is subsequently probed in an unbiased fashion with autologous patient serum to search for tumor proteins that elicit host immune responses.

**Syngeneic** The cells, tissues, and histocompatibility molecules derived from a genetically identical individual (cf. syngeneic, allogeneic), such as an identical twin, or fully inbred strains of laboratory animals.

**T Cells or T lymphocytes** Lymphocytes of the adaptive immune system that mature in the thymus from bone marrow-derived progenitors, which express clonally distributed antigen receptors, and which are stimulated by APC in secondary lymphoid organs to express different effector functions. The two major populations of T cells are based on the expression of CD4 or CD8 cell surface glycoproteins. CD4 T cells recognize peptide antigen associated with MHC class II molecules and are subdivided into subsets based largely on cytokine secretion profiles for the T-helper cells: T<sub>h</sub>1 (IFN- $\gamma$ ), T<sub>h</sub>2 (IL-4), and T<sub>h</sub>17 (IL-17). For T<sub>reg</sub>, the definition is based on a range of phenotypic markers (including Foxp3) and function (suppression of other immune cells). CD8 T cells recognize peptides bound to MHC class I molecules and generally have cytotoxic potential (see CTLs) but can also secrete cytokines (especially IFN- $\gamma$ ).

**T-Cell Receptor for antigen (TCR)** A highly variable receptor expressed exclusively by T cells, in a clonally distributed manner. It is not germline encoded but is generated by somatic rearrangement of gene segments. The ligand is a short peptide epitope bound to an MHC molecule expressed at the surface of another cell.

**Tolerance** Immunological tolerance refers to the unresponsiveness of T and B cells to a particular antigen. Central tolerance is established in the bone marrow for B cells and in the thymus for T cells, wherein cell highly reactive with self-antigens (autoreactive cells) can be eliminated. Residual populations of autoreactive cells that escape deletion are restrained by different mechanisms of peripheral tolerance. Tumor immunotherapy often aims to exploit these cells that may react to tumor expressed TAAs and may thus need to attempt to “break” tolerance.

**Tumor-associated antigens (TAAs).** These antigens are expressed by malignant cells but also by normal cells. They may still be useful targets for immunotherapy because tumor cells may express the TAA at a higher level than

normal tissue, or because normal cells express the TAA only at a certain stage of development, or in specific sites that may be shielded from immune attack (e.g., the so-called cancer-testis antigens). These antigens may be common to many patients, facilitating defined targeting and immune monitoring. However, since TAAs are self-antigens, it may be difficult to induce effective immune responses because of partial or complete immunological tolerance, and if this is overcome, there is a risk of inducing autoimmune pathology.

**Tumor-specific antigens (TSAs)** These antigens are only expressed by malignant cells (e.g., following mutations). They present advantages for immunotherapy because the patient should have no central tolerance to these antigens, and there is no risk of immune effector mechanisms damaging normal tissues because they do not express the antigen. However, many of these antigens are unique to individual patients, meaning that defined targeting and immune monitoring would need to be personalized.

## References

- Ali, S., King, G.D., Curtin, J.F., Candolfi, M., Xiong, W., Liu, C., Puntel, M., Cheng, Q., Prieto, J., Ribas, A., Kupiec-Weglinski, J., van Rooijen, N., Lassmann, H., Lowenstein, P.R., and Castro, M.G. (2005). Combined immunostimulation and conditional cytotoxic gene therapy provide long-term survival in a large glioma model. *Cancer Res* 65, 7194–204.
- Aloisi, F., Ria, F., and Adorini, L. (2000). Regulation of T-cell responses by CNS antigen-presenting cells: different roles for microglia and astrocytes. *Immunol Today* 21, 141–7.
- Andersen, M.H., Pedersen, L.O., Capeller, B., Brocker, E.B., Becker, J.C., and Thor, S.P. (2001). Spontaneous cytotoxic T-cell responses against survivin-derived MHC class I-restricted T-cell epitopes in situ as well as ex vivo in cancer patients. *Cancer Res* 61, 5964–68.
- Aoki, H., Mizuno, M., Natsume, A., Tsugawa, T., Tsujimura, K., Takahashi, T., and Yoshida, J. (2001). Dendritic cells pulsed with tumor extract-cationic liposome complex increase the induction of cytotoxic T lymphocytes in mouse brain tumor. *Cancer Immunol Immunother* 50, 463–8.
- Aulwurm, S., Wischhusen, J., Friese, M., Borst, J., and Weller, M. (2006). Immune stimulatory effects of CD70 override CD70-mediated immune cell apoptosis in rodent glioma models and confer long-lasting antiglioma immunity in vivo. *Int J Cancer* 118, 1728–35.
- Baldwin, N.G., Rice, C.D., Tuttle, T.M., Bear, H.D., Hirsch, J.I., and Merchant, R.E. (1997). Ex vivo expansion of tumor-draining lymph node cells using compounds which activate intracellular signal transduction. I. Characterization and in vivo anti-tumor activity of glioma-sensitized lymphocytes. *J Neurooncol* 32, 19–28.
- Bargou, R., Leo, E., Zugmaier, G., Klinger, M., Goebeler, M., Knop, S., Noppeney, R., Viardot, A., Hess, G., Schuler, M., Einsele, H., Brandl, C., Wolf, A., Kirchinger, P., Klappers, P., Schmidt, M., Riethmuller, G., Reinhardt, C., Baeuerle, P.A., and Kufer, P. (2008). Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. *Science* 321, 974–7.
- Barker, C.F. and Billingham, R.E. (1977). Immunologically privileged sites. *Adv Immunol* 25, 1–54.
- Barrat, F.J. and Coffman, R.L. (2008). Development of TLR inhibitors for the treatment of autoimmune diseases. *Immunol Rev* 223, 271–83.
- Barton, G.M. and Medzhitov, R. (2002). Toll-like receptors and their ligands. *Curr Top Microbiol Immunol* 270, 81–92.



- Battistini, L., Piccio, L., Rossi, B., Bach, S., Galgani, S., Gasperini, C., Ottoboni, L., Ciabini, D., Caramia, M.D., Bernardi, G., Laudanna, C., Scarpini, E., McEver, R.P., Butcher, E.C., Corsellino, G., and Constantin, G. (2003). CD8+ T cells from patients with acute multiple sclerosis display selective increase of adhesiveness in brain venules: a critical role for P-selectin glycoprotein ligand-1. *Blood* 101, 4775–82.
- Beauvillain, C., Donnou, S., Jarry, U., Scotet, M., Gascan, H., Delneste, Y., Guermontprez, P., Jeannin, P., and Couez, D. (2008). Neonatal and adult microglia cross-present exogenous antigens. *Glia* 56, 69–77.
- Bechmann, I., Galea, I., and Perry, V.H. (2007). What is the blood-brain barrier (not)? *Trends Immunol* 28, 5–11.
- Bigner, D.D., Pitts, O.M., and Wikstrand, C.J. (1981). Induction of lethal experimental allergic encephalomyelitis in nonhuman primates and guinea pigs with human glioblastoma multiforme tissue. *J Neurosurg* 55, 32–42.
- Birnbaum, T., Roider, J., Schankin, C.J., Padovan, C.S., Schichor, C., Goldbrunner, R., and Straube, A. (2007). Malignant gliomas actively recruit bone marrow stromal cells by secreting angiogenic cytokines. *J Neurooncol* 83, 241–7.
- Bodey, B., Bodey, V., and Siegel, S.E. (2008). Expression in childhood primary brain tumors of NY-ESO-1, a cancer/testis antigen: an immunohistochemical study. *In Vivo* 22, 83–7.
- Bodmer, S., Strommer, K., Frei, K., Siepl, C., de Tribolet, N., Heid, I., and Fontana, A. (1989). Immunosuppression and transforming growth factor-beta in glioblastoma. Preferential production of transforming growth factor-beta 2. *J Immunol* 143, 3222–9.
- Brabb, T., von Dassow, P., Ordonez, N., Schnabel, B., Duke, B., and Goverman, J. (2000). In situ tolerance within the central nervous system as a mechanism for preventing autoimmunity. *J Exp Med* 192, 871–80.
- Broder, H., Anderson, A., Kremen, T.J., Odesa, S.K., and Liao, L.M. (2003). MART-1 adenovirus-transduced dendritic cell immunization in a murine model of metastatic central nervous system tumor. *J Neurooncol* 64, 21–30.
- Brown, C.E., Vishwanath, R.P., Aguilar, B., Starr, R., Najbauer, J., Aboody, K.S., and Jensen, M.C. (2007). Tumor-derived chemokine MCP-1/CCL2 is sufficient for mediating tumor tropism of adoptively transferred T cells. *J Immunol* 179, 3332–41.
- Burnet F.M., (1970). The concept of immunological surveillance. *Prog Exp Tumor Res* 13, 1–27.
- Butcher, E.C., Williams, M., Youngman, K., Rott, L., and Briskin, M. (1999). Lymphocyte trafficking and regional immunity. *Adv Immunol* 72, 209–53.
- Butterfield, L.H., Meng, W.S., Koh, A., Vollmer, C.M., Ribas, A., Dissette, V.B., Faull, K., Glaspy, J.A., McBride, W.H., and Economou, J.S. (2001). T cell responses to HLA-A\*0201-restricted peptides derived from human alpha fetoprotein. *J Immunol* 166, 5300–8.
- Calzascia, T., Di Bernardino-Besson, W., Wilmotte, R., Masson, F., de Tribolet, N., Dietrich, P.Y., and Walker, P.R. (2003). Cutting edge: cross-presentation as a mechanism for efficient recruitment of tumor-specific CTL to the brain. *J Immunol* 171, 2187–91.
- Calzascia, T., Masson, F., Di Bernardino-Besson, W., Contassot, E., Wilmotte, R., Aurrand-Lions, M., Ruegg, C., Dietrich, P.Y., and Walker, P.R. (2005). Homing phenotypes of tumor-specific CD8 T cells are predetermined at the tumor site by crosspresenting APCs. *Immunity* 22, 175–84.
- Carson, M.J., Reilly, C.R., Sutcliffe, J.G., and Lo, D. (1999). Disproportionate recruitment of CD8+ T cells into the central nervous system by professional antigen-presenting cells. *Am J Pathol* 154, 481–94.
- Caruso, D.A., Orme, L.M., Neale, A.M., Radcliff, F.J., Amor, G.M., Maixner, W., Downie, P., Hassall, T.E., Tang, L.K., and Ashley, D.M. (2004). Results of a phase 1 study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children and young adults with brain cancer. *Neuro-Oncology* 6, 236–46.

- Cayrol, R., Wosik, K., Berard, J.L., Dodelet-Devillers, A., Ifergan, I., Kebir, H., Haqqani, A.S., Kreymborg, K., Krug, S., Moumdjian, R., Bouthillier, A., Becher, B., Arbour, N., David, S., Stanimirovic, D., and Prat, A. (2008). Activated leukocyte cell adhesion molecule promotes leukocyte trafficking into the central nervous system. *Nat Immunol* 9, 137–45.
- Chabalgoity, J.A., Dougan, G., Mastroeni, P., and Aspinall, R.J. (2002). Live bacteria as the basis for immunotherapies against cancer. *Expert Rev Vaccines* 1, 495–505.
- Chang, A.E., Shu, S.Y., Chou, T., Lafreniere, R., and Rosenberg, S.A. (1986). Differences in the effects of host suppression on the adoptive immunotherapy of subcutaneous and visceral tumors. *Cancer Res* 46, 3426–30.
- Chen, Y.T., Scanlan, M.J., Sahin, U., Tureci, O., Gure, A.O., Tsang, S., Williamson, B., Stockert, E., Pfreundschuh, M., and Old, L.J. (1997). A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc Natl Acad Sci U S A* 94, 1914–8.
- Chi, D.D., Merchant, R.E., Rand, R., Conrad, A.J., Garrison, D., Turner, R., Morton, D.L., and Hoon, D.S. (1997). Molecular detection of tumor-associated antigens shared by human cutaneous melanomas and gliomas. *Am J Pathol* 150, 2143–52.
- Ciesielski, M.J., Kozbor, D., Castanaro, C.A., Barone, T.A., and Fenstermaker, R.A. (2008). Therapeutic effect of a T helper cell supported CTL response induced by a survivin peptide vaccine against murine cerebral glioma. *Cancer Immunol Immunother.*
- Clairmont, C., Lee, K.C., Pike, J., Ittensohn, M., Low, K.B., Pawelek, J., Bermudes, D., Brecher, S.M., Margitich, D., Turnier, J., Li, Z., Luo, X., King, I., and Zheng, L.M. (2000). Biodistribution and genetic stability of the novel antitumor agent VNP20009, a genetically modified strain of *Salmonella typhimurium*. *J Infect Dis* 181, 1996–2002.
- Cobbs, C.S., Harkins, L., Samanta, M., Gillespie, G.Y., Bharara, S., King, P.H., Nabors, L.B., Cobbs, C.G., and Britt, W.J. (2002). Human cytomegalovirus infection and expression in human malignant glioma. *Cancer Res* 62, 3347–50.
- Csatary, L.K., Gosztonyi, G., Szeberenyi, J., Fabian, Z., Liszka, V., Bodey, B., and Csatary, C.M. (2004). MTH-68/H oncolytic viral treatment in human high-grade gliomas. *J Neurooncol* 67, 83–93.
- Cserr, H.F. and Knopf, P.M. (1992). Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. *Immunol Today* 13, 507–12.
- Curtin, J.F., Candolfi, M., Fakhouri, T.M., Liu, C., Alden, A., Edwards, M., Lowenstein, P.R., and Castro, M.G. (2008). Treg depletion inhibits efficacy of cancer immunotherapy: implications for clinical trials. *PLoS ONE* 3, e1983.
- Curtin, J.F., King, G.D., Candolfi, M., Greeno, R.B., Kroeger, K.M., Lowenstein, P.R., and Castro, M.G. (2005). Combining cytotoxic and immune-mediated gene therapy to treat brain tumors. *Curr Top Med Chem* 5, 1151–70.
- Davoust, N., Vuillat, C., Androdias, G., and Nataf, S. (2008). From bone marrow to microglia: barriers and avenues. *Trends Immunol* 29, 227–34.
- Desbaillets, I., Tada, M., de Tribolet, N., Diserens, A.-C., Hamou, M.-F., and Van Meir, E.G. (1994). Human astrocytomas and glioblastomas express Monocyte Chemoattractant Protein-1 (MCP-1) in vivo and in vitro. *Int J Cancer*, 58, 240–7.
- De Vleeschouwer, S., Fieuw, S., Rutkowski, S., Van Calenbergh, F., Van Loon, J., Goffin, J., Sciot, R., Wilms, G., Demaerel, P., Warmuth-Metz, M., Soerensen, N., Wolff, J.E., Wagner, S., Kaempgen, E., and Van Gool, S.W. (2008). Postoperative adjuvant dendritic cell-based immunotherapy in patients with relapsed glioblastoma multiforme. *Clin Cancer Res* 14, 3098–104.
- Dey, N., Durden, D.L., and Van Meir, E.G. (2006). Cytokine expression and signaling in CNS tumors. In *Cytokines and the CNS II*. Benveniste, E.N. and Ransohoff, R.M. (eds.), CRC Press. pp.193–242.
- Dijkstra, I.M., de Haas, A.H., Brouwer, N., Boddeke, H.W., and Biber, K. (2006). Challenge with innate and protein antigens induces CCR7 expression by microglia in vitro and in vivo. *Glia* 54, 861–72.

- Dillman, R.O., Duma, C.M., Schiltz, P.M., DePriest, C., Ellis, R.A., Okamoto, K., Beutel, L.D., De Leon, C., and Chico, S. (2004). Intracavitary placement of autologous lymphokine-activated killer (LAK) cells after resection of recurrent glioblastoma. *J Immunother* 27, 398–404.
- Disis, M.L., Gooley, T.A., Rinn, K., Davis, D., Piepkorn, M., Cheever, M.A., Knutson, K.L., and Schiffman, K. (2002). Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines. *J Clin Oncol* 20, 2624–32.
- Disis, M.L., Schiffman, K., Guthrie, K., Salazar, L.G., Knutson, K.L., Goodell, V., dela Rosa, C., and Cheever, M.A. (2004). Effect of dose on immune response in patients vaccinated with an her-2/neu intracellular domain protein-based vaccine. *J Clin Oncol* 22, 1916–25.
- Dudley, M.E., Wunderlich, J.R., Yang, J.C., Sherry, R.M., Topalian, S.L., Restifo, N.P., Royal, R.E., Kammula, U., White, D.E., Mavroukakis, S.A., Rogers, L.J., Gracia, G.J., Jones, S.A., Mangiameli, D.P., Pelletier, M.M., Gea-Banacloche, J., Robinson, M.R., Berman, D.M., Filie, A.C., Abati, A., and Rosenberg, S.A. (2005). Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* 23, 2346–57.
- Dunn, G.P., Koebel, C.M., and Schreiber, R.D. (2006). Interferons, immunity and cancer immunoediting. *Nat Rev Immunol* 6, 836–48.
- Ehteshami, M., Kabos, P., Gutierrez, M.A., Samoto, K., Black, K.L., and Yu, J.S. (2003). Intratumoral dendritic cell vaccination elicits potent tumoricidal immunity against malignant glioma in rats. *J Immunother* 26, 107–16.
- Eisele, G., Wischhusen, J., Mittelbronn, M., Meyermann, R., Waldhauer, I., Steinle, A., Weller, M., and Friese, M.A. (2006). TGF-beta and metalloproteinases differentially suppress NKG2D ligand surface expression on malignant glioma cells. *Brain* 129, 2416–25.
- El Andaloussi, A., Han, Y., and Lesniak, M.S. (2006). Prolongation of survival following depletion of CD4+CD25+ regulatory T cells in mice with experimental brain tumors. *J Neurosurg* 105, 430–7.
- El Andaloussi, A. and Lesniak, M.S. (2007). CD4+ CD25+ FoxP3+ T-cell infiltration and heme oxygenase-1 expression correlate with tumor grade in human gliomas. *J Neurooncol* 83, 145–52.
- Engelhardt B. (2006). Molecular mechanisms involved in T cell migration across the blood-brain barrier. *J Neural Transm* 113, 477–85.
- Facoetti, A., Nano, R., Zelini, P., Morbini, P., Benericetti, E., Ceroni, M., Campoli, M., and Ferrone, S. (2005). Human leukocyte antigen and antigen processing machinery component defects in astrocytic tumors. *Clin Cancer Res* 11, 8304–11.
- Farina, C., Aloisi, F., and Meinl, E. (2007). Astrocytes are active players in cerebral innate immunity. *Trends Immunol* 28, 138–45.
- Fecci, P.E., Mitchell, D.A., Whitesides, J.F., Xie, W., Friedman, A.H., Archer, G.E., Herndon, J.E., 2nd, Bigner, D.D., Dranoff, G., and Sampson, J.H. (2006a). Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. *Cancer Res* 66, 3294–302.
- Fecci, P.E., Ochiai, H., Mitchell, D.A., Grossi, P.M., Sweeney, A.E., Archer, G.E., Cummings, T., Allison, J.P., Bigner, D.D., and Sampson, J.H. (2007). Systemic CTLA-4 blockade ameliorates glioma-induced changes to the CD4+ T cell compartment without affecting regulatory T-cell function. *Clin Cancer Res* 13, 2158–67.
- Fecci, P.E., Sweeney, A.E., Grossi, P.M., Nair, S.K., Learn, C.A., Mitchell, D.A., Cui, X., Cummings, T.J., Bigner, D.D., Gilboa, E., and Sampson, J.H. (2006b). Systemic anti-CD25 monoclonal antibody administration safely enhances immunity in murine glioma without eliminating regulatory T cells. *Clin Cancer Res* 12, 4294–305.
- Fischer, H.G. and Reichmann, G. (2001). Brain dendritic cells and macrophages/microglia in central nervous system inflammation. *J Immunol* 166, 2717–26.

- Freije, W.A., Castro-Vargas, F.E., Fang, Z., Horvath, S., Cloughesy, T., Liau, L.M., Mischel, P.S., and Nelson, S.F. (2004). Gene expression profiling of gliomas strongly predicts survival. *Cancer Res* 64, 6503–10.
- Fujita, M., Zhu, X., Sasaki, K., Ueda, R., Low, K.L., Pollack, I.F., and Okada, H. (2008). Inhibition of STAT3 promotes the efficacy of adoptive transfer therapy using type-1 CTLs by modulation of the immunological microenvironment in a murine intracranial glioma. *J Immunol* 180, 2089–98.
- Galea, I., Bernardes-Silva, M., Forse, P.A., van Rooijen, N., Liblau, R.S., and Perry, V.H. (2007). An antigen-specific pathway for CD8 T cells across the blood-brain barrier. *J Exp Med* 204, 2023–30.
- Gattinoni, L., Finkelstein, S.E., Klebanoff, C.A., Antony, P.A., Palmer, D.C., Spiess, P.J., Hwang, L.N., Yu, Z., Wrzesinski, C., Heimann, D.M., Surh, C.D., Rosenberg, S.A., and Restifo, N.P. (2005). Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. *J Exp Med* 202, 907–12.
- Glick, R.P., Lichter, T., and Cohen, E.P. (2001). Cytokine-based immuno-gene therapy for brain tumors. In *Brain Tumor Immunotherapy*, L.M. Liau, Becker, D.P., Cloughesy, T.F., and Bigner, D.D., eds. (Totowa, New Jersey: Humana Press), pp. 273–88.
- Graeber, M.B., Scheithauer, B.W., and Kreutzberg, G.W. (2002). Microglia in brain tumors. *Glia* 40, 252–9.
- Grauer, O.M., Nierkens, S., Bennink, E., Toonen, L.W., Boon, L., Wesseling, P., Suttmuller, R.P., and Adema, G.J. (2007). CD4+ FoxP3+ regulatory T cells gradually accumulate in gliomas during tumor growth and efficiently suppress antiglioma immune responses in vivo. *Int J Cancer* 121, 95–105.
- Grauer, O.M., Suttmuller, R.P., van Maren, W., Jacobs, J.F., Bennink, E., Toonen, L.W., Nierkens, S., and Adema, G.J. (2008). Elimination of regulatory T cells is essential for an effective vaccination with tumor lysate-pulsed dendritic cells in a murine glioma model. *Int J Cancer* 122, 1794–802.
- Hanisch, U.K. and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci* 10, 1387–94.
- Hatano, M., Kuwashima, N., Tatsumi, T., Dusak, J.E., Nishimura, F., Reilly, K.M., Storkus, W.J., and Okada, H. (2004). Vaccination with EphA2-derived T cell-epitopes promotes immunity against both EphA2-expressing and EphA2-negative tumors. *J Transl Med* 2, 40.
- Hatterer, E., Davoust, N., Didier-Bazes, M., Vuailat, C., Malcus, C., Belin, M.F., and Nataf, S. (2006). How to drain without lymphatics? Dendritic cells migrate from the cerebrospinal fluid to the B-cell follicles of cervical lymph nodes. *Blood* 107, 806–12.
- Hayes, R.L., Koslow, M., Hiesiger, E.M., Hymes, K.B., Hochster, H.S., Moore, E.J., Pierz, D.M., Chen, D.K., Budzilovich, G.N., and Ransohoff, J. (1995). Improved long term survival after intracavitary interleukin-2 and lymphokine-activated killer cells for adults with recurrent malignant glioma. *Cancer* 76, 840–52.
- Hazelrigg, M.R., Hirsch, J.I., and Merchant, R.E. (2002). Distribution of adoptively transferred, tumor-sensitized lymphocytes in the glioma-bearing rat. *J Neurooncol* 60, 143–50.
- Heimberger, A.B., Abou-Ghazal, M., Reina-Ortiz, C., Yang, D.S., Sun, W., Qiao, W., Hiraoka, N., and Fuller, G.N. (2008). Incidence and Prognostic Impact of FoxP3+ Regulatory T Cells in Human Gliomas. *Clin Cancer Res* 14, 5166–72.
- Heimberger, A.B., Archer, G.E., Crotty, L.E., McLendon, R.E., Friedman, A.H., Friedman, H.S., Bigner, D.D., and Sampson, J.H. (2002). Dendritic cells pulsed with a tumor-specific peptide induce long-lasting immunity and are effective against murine intracerebral melanoma. *Neurosurgery* 50, 158–64; discussion 64–6.
- Heimberger, A.B., Crotty, L.E., Archer, G.E., Hess, K.R., Wikstrand, C.J., Friedman, A.H., Friedman, H.S., Bigner, D.D., and Sampson, J.H. (2003). Epidermal growth factor receptor VIII peptide vaccination is efficacious against established intracerebral tumors. *Clin Cancer Res* 9, 4247–54.

- Hickey W.F. (1999). Leukocyte traffic in the central nervous system: the participants and their roles. *Semin Immunol* 11, 125–37.
- Horvath, S., Zhang, B., Carlson, M., Lu, K.V., Zhu, S., Felciano, R.M., Laurance, M.F., Zhao, W., Qi, S., Chen, Z., Lee, Y., Scheck, A.C., Liau, L.M., Wu, H., Geschwind, D.H., Febbo, P.G., Kornblum, H.I., Cloughesy, T.F., Nelson, S.F., and Mischel, P.S. (2006). Analysis of oncogenic signaling networks in glioblastoma identifies ASPM as a molecular target. *Proc Natl Acad Sci U S A* 103, 17402–7.
- Husain, S.R., Joshi, B.H., and Puri, R.K. (2001). Interleukin-13 receptor as a unique target for anti-glioblastoma therapy. *Int J Cancer* 92, 168–75.
- Hussain, S.F., Yang, D., Suki, D., Aldape, K., Grimm, E., and Heimberger, A.B. (2006). The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. *Neuro Oncol* 8, 261–79.
- Insug, O., Ku, G., Ertl, H.C., and Blaszczyk-Thurin, M. (2002). A dendritic cell vaccine induces protective immunity to intracranial growth of glioma. *Anticancer Res* 22, 613–21.
- Ishikawa, E., Tsuboi, K., Saijo, K., Harada, H., Takano, S., Nose, T., and Ohno, T. (2004). Autologous natural killer cell therapy for human recurrent malignant glioma. *Anticancer Res* 24, 1861–71.
- Jordan, J.T., Sun, W., Hussain, S.F., DeAngulo, G., Prabhu, S.S., and Heimberger, A.B. (2008). Preferential migration of regulatory T cells mediated by glioma-secreted chemokines can be blocked with chemotherapy. *Cancer Immunol Immunother* 57, 123–31.
- Jouanneau, E., Poujol, D., Gulia, S., Le Mercier, I., Blay, J.Y., Belin, M.F., and Puisieux, I. (2006). Dendritic cells are essential for priming but inefficient for boosting antitumor immune response in an orthotopic murine glioma model. *Cancer Immunol Immunother* 55, 254–67.
- Jung, S., Unutmaz, D., Wong, P., Sano, G., De los Santos, K., Sparwasser, T., Wu, S., Vuthoori, S., Ko, K., Zavala, F., Pamer, E.G., Littman, D.R., and Lang, R.A. (2002). In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. *Immunity* 17, 211–20.
- Karman, J., Chu, H.H., Co, D.O., Seroogy, C.M., Sandor, M., and Fabry, Z. (2006). Dendritic cells amplify T cell-mediated immune responses in the central nervous system. *J Immunol* 177, 7750–60.
- Karman, J., Ling, C., Sandor, M., and Fabry, Z. (2004). Initiation of immune responses in brain is promoted by local dendritic cells. *J Immunol* 173, 2353–61.
- Katoh, M., Wilmotte, R., Belkouch, M.C., de Tribolet, N., Pizzolato, G., and Dietrich, P.Y. (2003). Survivin in brain tumors: an attractive target for immunotherapy. *J Neurooncol* 64, 71–6.
- Kiertscher, S.M. and Roth, M.D. (1996). Human CD14+ leukocytes acquire the phenotype and function of antigen-presenting dendritic cells when cultured in GM-CSF and IL-4. *J Leukoc Biol* 59, 208–18.
- Kikuchi, T., Akasaki, Y., Abe, T., Fukuda, T., Saotome, H., Ryan, J.L., Kufe, D.W., and Ohno, T. (2004). Vaccination of glioma patients with fusions of dendritic and glioma cells and recombinant human interleukin 12. *J Immunother* 27, 452–9.
- Kikuchi, T., Akasaki, Y., Abe, T., and Ohno, T. (2002). Intratumoral injection of dendritic and irradiated glioma cells induces anti-tumor effects in a mouse brain tumor model. *Cancer Immunol Immunother* 51, 424–30.
- Kikuchi, T., Akasaki, Y., Irie, M., Homma, S., Abe, T., and Ohno, T. (2001). Results of a phase I clinical trial of vaccination of glioma patients with fusions of dendritic and glioma cells. *Cancer Immunol Immunother* 50, 337–44.
- Kim, C.H., Hong, M.J., Park, S.D., Kim, C.K., Park, M.Y., Sohn, H.J., Cho, H.I., Kim, T.G., and Hong, Y.K. (2006). Enhancement of anti-tumor immunity specific to murine glioma by vaccination with tumor cell lysate-pulsed dendritic cells engineered to produce interleukin-12. *Cancer Immunol Immunother* 55, 1309–19.

- Kjaergaard, J., Wang, L.X., Kuriyama, H., Shu, S., and Plautz, G.E. (2005). Active immunotherapy for advanced intracranial murine tumors by using dendritic cell-tumor cell fusion vaccines. *J Neurosurg* 103, 156–64.
- Klebanoff, C.A., Gattinoni, L., Torabi-Parizi, P., Kerstann, K., Cardones, A.R., Finkelstein, S.E., Palmer, D.C., Antony, P.A., Hwang, S.T., Rosenberg, S.A., Waldmann, T.A., and Restifo, N.P. (2005). Central memory self/tumor-reactive CD8 + T cells confer superior antitumor immunity compared with effector memory T cells. *Proc Natl Acad Sci U S A* 102, 9571–6.
- Knutson, K.L. and Disis, M.L. (2001). Expansion of HER2/neu-specific T cells ex vivo following immunization with a HER2/neu peptide-based vaccine. *Clin Breast Cancer* 2, 73–9.
- Knutson, K.L., Schiffman, K., and Disis, M.L. (2001). Immunization with a HER-2/neu helper peptide vaccine generates HER-2/neu CD8 T-cell immunity in cancer patients. *J Clin Invest* 107, 477–84.
- Kruse, C.A., Cepeda, L., Owens, B., Johnson, S.D., Stears, J., and Lillehei, K.O. (1997). Treatment of recurrent glioma with intracavitary alloreactive cytotoxic T lymphocytes and interleukin-2. *Cancer Immunol Immunother* 45, 77–87.
- Kruse, C.A., Lillehei, K.O., Mitchell, D.H., Kleinschmidt-DeMasters, B., and Bellgrau, D. (1990). Analysis of interleukin 2 and various effector cell populations in adoptive immunotherapy of 9L rat gliosarcoma: allogeneic cytotoxic T lymphocytes prevent tumor take. *Proc Natl Acad Sci U S A* 87, 9577–81.
- Kuppner, M.C., Hamou, M.F., and de Tribolet, N. (1990). Activation and adhesion molecule expression on lymphoid infiltrates in human glioblastomas. *J Neuroimmunol* 29, 229–38.
- Kuwashima, N., Nishimura, F., Eguchi, J., Sato, H., Hatano, M., Tsugawa, T., Sakaida, T., Dusak, J.E., Fellows-Mayle, W.K., Papworth, G.D., Watkins, S.C., Gambotto, A., Pollack, I.F., Storkus, W.J., and Okada, H. (2005). Delivery of dendritic cells engineered to secrete IFN- $\alpha$  into central nervous system tumors enhances the efficacy of peripheral tumor cell vaccines: dependence on apoptotic pathways. *J Immunol* 175, 2730–40.
- Lanier L.L., (2008). Up on the tightrope: natural killer cell activation and inhibition. *Nat Immunol* 9, 495–502.
- Learn, C.A., Fecci, P.E., Schmittling, R.J., Xie, W., Karikari, I., Mitchell, D.A., Archer, G.E., Wei, Z., Dressman, H., and Sampson, J.H. (2006). Profiling of CD4+, CD8+, and CD4+CD25+CD45RO+FoxP3+ T cells in patients with malignant glioma reveals differential expression of the immunologic transcriptome compared with T cells from healthy volunteers. *Clin Cancer Res* 12, 7306–15.
- Liau, L.M., Black, K.L., Prins, R.M., Sykes, S.N., DiPatre, P.L., Cloughesy, T.F., Becker, D.P., and Bronstein, J.M. (1999). Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. *J Neurosurg* 90, 1115–24.
- Liau, L.M., Jensen, E.R., Kremen, T.J., Odesa, S.K., Sykes, S.N., Soung, M.C., Miller, J.F., and Bronstein, J.M. (2002). Tumor immunity within the central nervous system stimulated by recombinant *Listeria monocytogenes* vaccination. *Cancer Res* 62, 2287–93.
- Liau, L.M., Lallone, R.L., Seitz, R.S., Buznikov, A., Gregg, J.P., Kornblum, H.I., Nelson, S.F., and Bronstein, J.M. (2000). Identification of a human glioma-associated growth factor gene, granulin, using differential immuno-absorption. *Cancer Res* 60, 1353–60.
- Liau, L.M., Prins, R.M., Kiertscher, S.M., Odesa, S.K., Kremen, T.J., Giovannone, A.J., Lin, J.W., Chute, D.J., Mischel, P.S., Cloughesy, T.F., and Roth, M.D. (2005). Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. *Clin Cancer Res* 11, 5515–25.
- Ling, C., Verbny, Y.I., Banks, M.I., Sandor, M., and Fabry, Z. (2008). In situ activation of antigen-specific CD8(+) T cells in the presence of antigen in organotypic brain slices. *J Immunol* 180, 8393–9.
- Liu, G., Ying, H., Zeng, G., Wheeler, C.J., Black, K.L., and Yu, J.S. (2004a). HER-2, gp100, and MAGE-1 are expressed in human glioblastoma and recognized by cytotoxic T cells. *Cancer Res* 64, 4980–6.

- Liu, G., Yu, J.S., Zeng, G., Yin, D., Xie, D., Black, K.L., and Ying, H. (2004b). AIM-2: a novel tumor antigen is expressed and presented by human glioma cells. *J Immunother* 27, 220–6.
- Louis D.N. (2006). Molecular pathology of malignant gliomas. *Annu Rev Pathol* 1, 97–117.
- Luster, A.D., Alon, R., and von Andrian, U.H. (2005). Immune cell migration in inflammation: present and future therapeutic targets. *Nat Immunol* 6, 1182–90.
- Maecker, B., von, B.-B., Anderson, K.S., Vonderheide, R.H., and Schultze, J.L. (2001). Linking genomics to immunotherapy by reverse immunology–‘immunomics’ in the new millennium. *Curr Mol Med* 1, 609–19.
- Markovic, D.S., Glass, R., Synowitz, M., Rooijen, N., and Kettenmann, H. (2005). Microglia stimulate the invasiveness of glioma cells by increasing the activity of metalloprotease-2. *J Neuropathol Exp Neurol* 64, 754–62.
- Marx J. (2008). Cancer immunology. Cancer’s bulwark against immune attack: MDS cells. *Science* 319, 154–6.
- Masson, F., Calzascia, T., Di Bernardino-Besson, W., de Tribolet, N., Dietrich, P.Y., and Walker, P.R. (2007). Brain microenvironment promotes the final functional maturation of tumor-specific effector CD8+ T cells. *J Immunol* 179, 845–53.
- Matzinger P. (1994). Tolerance, danger, and the extended family. *Annu Rev Immunol* 12, 991–1045.
- Medewar P.B. (1948). Immunity to homologous grafted skin. III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue and to the anterior chamber of the eye. *Br J Exp Pathol* 29, 58–69.
- Mehling, M., Simon, P., Mittelbronn, M., Meyermann, R., Ferrone, S., Weller, M., and Wiendl, H. (2007). WHO grade associated downregulation of MHC class I antigen-processing machinery components in human astrocytomas: does it reflect a potential immune escape mechanism? *Acta Neuropathol* 114, 111–9.
- Merchant, R.E., Baldwin, N.G., Rice, C.D., and Bear, H.D. (1997). Adoptive immunotherapy of malignant glioma using tumor-sensitized T lymphocytes. *Neurol Res* 19, 145–52.
- Merchant, R.E., Grant, A.J., Merchant, L.H., and Young, H.F. (1988a). Adoptive immunotherapy for recurrent glioblastoma multiforme using lymphokine activated killer cells and recombinant interleukin-2. *Cancer* 62, 665–71.
- Merchant, R.E., Merchant, L.H., Cook, S.H., McVicar, D.W., and Young, H.F. (1988b). Intraleisional infusion of lymphokine-activated killer (LAK) cells and recombinant interleukin-2 (rIL-2) for the treatment of patients with malignant brain tumor. *Neurosurgery* 23, 725–32.
- Mischel, P.S., Nelson, S.F., and Cloughesy, T.F. (2003a). Molecular analysis of glioblastoma: pathway profiling and its implications for patient therapy. *Cancer Biol Ther* 2, 242–7.
- Mischel, P.S., Shai, R., Shi, T., Horvath, S., Lu, K.V., Choe, G., Seligson, D., Kremen, T.J., Palotie, A., Liau, L.M., Cloughesy, T.F., and Nelson, S.F. (2003b). Identification of molecular subtypes of glioblastoma by gene expression profiling. *Oncogene* 22, 2361–73.
- Mitchell, D.A., Fecci, P.E., and Sampson, J.H. (2003). Adoptive immunotherapy for malignant glioma. *Cancer J* 9, 157–66.
- Mitchell, D.A., Xie, W., Schmittling, R., Learn, C., Friedman, A., McLendon, R.E., and Sampson, J.H. (2008). Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma. *Neuro Oncol* 10, 10–8.
- Morgan, R.A., Dudley, M.E., Wunderlich, J.R., Hughes, M.S., Yang, J.C., Sherry, R.M., Royal, R.E., Topalian, S.L., Kammula, U.S., Restifo, N.P., Zheng, Z., Nahvi, A., de Vries, C.R., Rogers-Freezer, L.J., Mavroukakis, S.A., and Rosenberg, S.A. (2006). Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* 314, 126–9.
- Morse, M.A., Clay, T.M., Hobeika, A.C., Osada, T., Khan, S., Chui, S., Niedzwiecki, D., Panicali, D., Schlom, J., and Lyerly, H.K. (2005). Phase I study of immunization with dendritic cells modified with fowlpox encoding carcinoembryonic antigen and costimulatory molecules. *Clin Cancer Res* 11, 3017–24.

- Murphy, G.P., Barren, R.J., Erickson, S.J., Bowes, V.A., Wolfert, R.L., Bartsch, G., Klocker, H., Pointner, J., Reissigl, A., McLeod, D.G., Douglas, T., Morgan, T., Kenny, G.M., Ragde, H., Boynton, A.L., and Holmes, E.H. (1996). Evaluation and comparison of two new prostate carcinoma markers. Free-prostate specific antigen and prostate specific membrane antigen. *Cancer* 78, 809–18.
- Murphy, G.P., Tjoa, B.A., Simmons, S.J., Jarisch, J., Bowes, V.A., Ragde, H., Rogers, M., Elgamal, A., Kenny, G.M., Cobb, O.E., Ireton, R.C., Troychak, M.J., Salgaller, M.L., and Boynton, A.L. (1999). Infusion of dendritic cells pulsed with HLA-A2-specific prostate-specific membrane antigen peptides: a phase II prostate cancer vaccine trial involving patients with hormone-refractory metastatic disease. *Prostate* 38, 73–8.
- Ni, H.T., Spellman, S.R., Jean, W.C., Hall, W.A., and Low, W.C. (2001). Immunization with dendritic cells pulsed with tumor extract increases survival of mice bearing intracranial gliomas. *J Neurooncol* 51, 1–9.
- Nishimura, F., Dusak, J.E., Eguchi, J., Zhu, X., Gambotto, A., Storkus, W.J., and Okada, H. (2006). Adoptive transfer of type 1 CTL mediates effective anti-central nervous system tumor response: critical roles of IFN-inducible protein-10. *Cancer Res* 66, 4478–87.
- Okada, H., Attanucci, J., Giezeman-Smits, K.M., Brissette-Storkus, C., Fellows, W.K., Gambotto, A., Pollack, I.F., Pogue-Geile, K., Lotze, M.T., Bozik, M.E., and Chambers, W.H. (2001). Immunization with an antigen identified by cytokine tumor vaccine-assisted SEREX (CAS) suppressed growth of the rat 9L glioma in vivo. *Cancer Res* 61, 2625–31.
- Okada, H., Lieberman, F.S., Edington, H.D., Witham, T.F., Wargo, M.J., Cai, Q., Elder, E.H., Whiteside, T.L., Schold, S.C., Jr., and Pollack, I.F. (2003). Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of recurrent glioblastoma: preliminary observations in a patient with a favorable response to therapy. *J Neurooncol* 64, 13–20.
- Okada, H., Low, K.L., Kohanbash, G., McDonald, H.A., Hamilton, R.L., and Pollack, I.F. (2008). Expression of glioma-associated antigens in pediatric brain stem and non-brain stem gliomas. *J Neurooncol* 88, 245–50.
- Okada, H. and Pollack, I.F. (2004). Cytokine gene therapy for malignant glioma. *Expert Opin Biol Ther* 4, 1609–20.
- Okano, F., Storkus, W.J., Chambers, W.H., Pollack, I.F., and Okada, H. (2002). Identification of a novel HLA-A\*0201-restricted, cytotoxic T lymphocyte epitope in a human glioma-associated antigen, interleukin 13 receptor alpha2 chain. *Clin Cancer Res* 8, 2851–5.
- Pallasch, C.P., Struss, A.K., Munnia, A., Konig, J., Steudel, W.I., Fischer, U., and Meese, E. (2005). Autoantibodies against GLEA2 and PHF3 in glioblastoma: tumor-associated autoantibodies correlated with prolonged survival. *Int J Cancer* 117, 456–9.
- Panelli, M.C., Wunderlich, J., Jeffries, J., Wang, E., Mixon, A., Rosenberg, S.A., and Marincola, F.M. (2000). Phase I study in patients with metastatic melanoma of immunization with dendritic cells presenting epitopes derived from the melanoma-associated antigens MART-1 and gp100. *J Immunother* 23, 487–98.
- Parkhurst, M.R., Fitzgerald, E.B., Southwood, S., Sette, A., Rosenberg, S.A., and Kawakami, Y. (1998). Identification of a shared HLA-A\*0201-restricted T-cell epitope from the melanoma antigen tyrosinase-related protein 2 (TRP2). *Cancer Res* 58, 4895–901.
- Parkhurst, M.R., Salgaller, M.L., Southwood, S., Robbins, P.F., Sette, A., Rosenberg, S.A., and Kawakami, Y. (1996). Improved induction of melanoma-reactive CTL with peptides from the melanoma antigen gp100 modified at HLA-A\*0201-binding residues. *J Immunol* 157, 2539–48.
- Parsa, A.T., Waldron, J.S., Panner, A., Crane, C.A., Parney, I.F., Barry, J.J., Cachola, K.E., Murray, J.C., Tihan, T., Jensen, M.C., Mischel, P.S., Stokoe, D., and Pieper, R.O. (2007). Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat Med* 13, 84–8.
- Pellegatta, S., Poliani, P.L., Corno, D., Grisoli, M., Cusimano, M., Ubiali, F., Baggi, F., Bruzzone, M.G., and Finocchiaro, G. (2006). Dendritic cells pulsed with glioma lysates



- induce immunity against syngeneic intracranial gliomas and increase survival of tumor-bearing mice. *Neurol Res* 28, 527–31.
- Peng, L., Kjaergaard, J., Plautz, G.E., Awad, M., Drazba, J.A., Shu, S., and Cohen, P.A. (2002). Tumor-induced L-selectinhigh suppressor T cells mediate potent effector T cell blockade and cause failure of otherwise curative adoptive immunotherapy. *J Immunol* 169, 4811–21.
- Platten, M. and Steinman, L. (2005). Multiple sclerosis: trapped in deadly glue. *Nat Med* 11, 252–3.
- Plautz, G.E., Miller, D.W., Barnett, G.H., Stevens, G.H., Maffett, S., Kim, J., Cohen, P.A., and Shu, S. (2000). T cell adoptive immunotherapy of newly diagnosed gliomas. *Clin Cancer Res* 6, 2209–18.
- Plautz, G.E., Touhalisky, J.E., and Shu, S. (1997). Treatment of murine gliomas by adoptive transfer of ex vivo activated tumor-draining lymph node cells. *Cell Immunol* 178, 101–7.
- Prins, R.M., Bruhn, K.W., Craft, N., Lin, J.W., Kim, C.H., Odesa, S.K., Miller, J.F., and Liao, L.M. (2006a). Central nervous system tumor immunity generated by a recombinant listeria monocytogenes vaccine targeting tyrosinase related protein-2 and real-time imaging of intracranial tumor burden. *Neurosurgery* 58, 169–78; discussion 69–78.
- Prins, R.M., Cloughesy, T.F., and Liao, L.M. (2008a). Cytomegalovirus immunity after vaccination with autologous glioblastoma lysate. *N Engl J Med* 359, 539–41.
- Prins, R.M., Craft, N., Bruhn, K.W., Khan-Farooqi, H., Koya, R.C., Stripecke, R., Miller, J.F., and Liao, L.M. (2006b). The TLR-7 agonist, imiquimod, enhances dendritic cell survival and promotes tumor antigen-specific T cell priming: relation to central nervous system antitumor immunity. *J Immunol* 176, 157–64.
- Prins, R.M., Odesa, S.K., and Liao, L.M. (2003). Immunotherapeutic targeting of shared melanoma-associated antigens in a murine glioma model. *Cancer Res* 63, 8487–91.
- Prins, R.M., Scott, G.P., Merchant, R.E., and Graf, M.R. (2002). Irradiated tumor cell vaccine for treatment of an established glioma. II. Expansion of myeloid suppressor cells that promote tumor progression. *Cancer Immunol Immunother* 51, 190–9.
- Prins, R.M., Shu, C.J., Radu, C.G., Vo, D.D., Khan-Farooqi, H., Soto, H., Yang, M.Y., Lin, M.S., Shelly, S., Witte, O.N., Ribas, A., and Liao, L.M. (2008b). Anti-tumor activity and trafficking of self, tumor-specific T cells against tumors located in the brain. *Cancer Immunol Immunother* 57, 1279–89.
- Prins, R.M., Vo, D.D., Khan-Farooqi, H., Yang, M.Y., Soto, H., Economou, J.S., Liao, L.M., and Ribas, A. (2006c). NK and CD4 cells collaborate to protect against melanoma tumor formation in the brain. *J Immunol* 177, 8448–55.
- Pulendran B., (2005). Variiegation of the immune response with dendritic cells and pathogen recognition receptors. *J Immunol* 174, 2457–65.
- Qin, Z., Schwartzkopff, J., Pradera, F., Kammertoens, T., Seliger, B., Pircher, H., and Blankenstein, T. (2003). A critical requirement of interferon gamma-mediated angiostasis for tumor rejection by CD8+ T cells. *Cancer Res* 63, 4095–100.
- Quattrocchi, K.B., Miller, C.H., Cush, S., Bernard, S.A., Dull, S.T., Smith, M., Gudeman, S., and Varia, M.A. (1999). Pilot study of local autologous tumor infiltrating lymphocytes for the treatment of recurrent malignant gliomas. *J Neurooncol* 45, 141–57.
- Rebenko-Moll, N.M., Liu, L., Cardona, A., and Ransohoff, R.M. (2006). Chemokines, mononuclear cells and the nervous system: heaven (or hell) is in the details. *Curr Opin Immunol* 18, 683–9.
- Ribas, A., (2006). Update on immunotherapy for melanoma. *J Natl Compr Canc Netw* 4, 687–94.
- Ribas, A., Butterfield, L.H., Hu, B., Dissette, V.B., Chen, A.Y., Koh, A., Amarnani, S.N., Glaspy, J.A., McBride, W.H., and Economou, J.S. (2000). Generation of T-cell immunity to a murine melanoma using MART-1-engineered dendritic cells. *J Immunother* 23, 59–66.
- Rice, C.D., Baldwin, N.G., Biron, R.T., Bear, H.D., and Merchant, R.E. (1997). Ex vivo expansion of tumor-draining lymph node cells using compounds which activate

- intracellular signal transduction. II. Cytokine production and in vivo efficacy of glioma-sensitized lymphocytes. *J Neurooncol* 32, 29–38.
- Rosenberg, S.A., Spiess, P.J., and Kleiner, D.E. (2002). Antitumor effects in mice of the intravenous injection of attenuated *Salmonella typhimurium*. *J Immunother* 25, 218–25.
- Roth, P., Mittelbronn, M., Wick, W., Meyermann, R., Tatagiba, M., and Weller, M. (2007). Malignant glioma cells counteract antitumor immune responses through expression of lectin-like transcript-1. *Cancer Res* 67, 3540–4.
- Ryu, J., Kim, C., Cheong, J., Bak, K., Kim, J., and Oh, S. (2006). Co-expression of survivin and Bcl-2 in primary brain tumors: their potential effect on anti-apoptosis. *J Korean Neurosurg Soc* 40, 1–5.
- Sahin, U., Koslowski, M., Tureci, O., Eberle, T., Zwick, C., Romeike, B., Moringlane, J.R., Schwechheimer, K., Feiden, W., and Pfreundschuh, M. (2000). Expression of cancer testis genes in human brain tumors. *Clin Cancer Res* 6, 3916–22.
- Saikali, S., Avril, T., Collet, B., Hamlat, A., Bansard, J.Y., Drenou, B., Guegan, Y., and Quillien, V. (2007). Expression of nine tumour antigens in a series of human glioblastoma multiforme: interest of EGFRvIII, IL-13Ralpha2, gp100 and TRP-2 for immunotherapy. *J Neurooncol* 81, 139–48.
- Sakaguchi, S., Yamaguchi, T., Nomura, T., and Ono, M. (2008). Regulatory T cells and immune tolerance. *Cell* 133, 775–87.
- Salgaller, M.L. (2000). American Association for Cancer Research: dendritic cells: strategies and vaccines. April 1–5, 2000, San Francisco, CA, USA. *Expert Opin Investig Drugs* 9, 1407–12.
- Salgaller, M.L. and Liau, L.M. (2006). Current status of clinical trials for glioblastoma. *Reviews on Recent Clinical Trials* 1, 265–81.
- Sasaki, K., Zhu, X., Vasquez, C., Nishimura, F., Dusak, J.E., Huang, J., Fujita, M., Wesa, A., Potter, D.M., Walker, P.R., Storkus, W.J., and Okada, H. (2007). Preferential expression of very late antigen-4 on type 1 CTL cells plays a critical role in trafficking into central nervous system tumors. *Cancer Res* 67, 6451–8.
- Schmits, R., Cochlovius, B., Treitz, G., Regitz, E., Ketter, R., Preuss, K.D., Romeike, B.F., and Pfreundschuh, M. (2002). Analysis of the antibody repertoire of astrocytoma patients against antigens expressed by gliomas. *Int J Cancer* 98, 73–7.
- Schneider, T., Gerhards, R., Kirches, E., and Firsching, R. (2001). Preliminary results of active specific immunization with modified tumor cell vaccine in glioblastoma multiforme. *J Neurooncol* 53, 39–46.
- Shimato, S., Natsume, A., Wakabayashi, T., Tsujimura, K., Nakahara, N., Ishii, J., Ito, M., Akatsuka, Y., Kuzushima, K., and Yoshida, J. (2008). Identification of a human leukocyte antigen-A24-restricted T-cell epitope derived from interleukin-13 receptor alpha2 chain, a glioma-associated antigen. *J Neurosurg* 109, 117–22.
- Siesjo, P., Visse, E., and Sjogren, H.O. (1996). Cure of established, intracerebral rat gliomas induced by therapeutic immunizations with tumor cells and purified APC or adjuvant IFN-gamma treatment. *J Immunother Emphasis Tumor Immunol* 19, 334–45.
- Sitkovsky, M. and Lukashev, D. (2005). Regulation of immune cells by local-tissue oxygen tension: HIF1 alpha and adenosine receptors. *Nat Rev Immunol* 5, 712–21.
- Steiner, H.H., Bonsanto, M.M., Beckhove, P., Brysch, M., Geletneky, K., Ahmadi, R., Schuele-Freyer, R., Kremer, P., Ranaie, G., Matejic, D., Bauer, H., Kiessling, M., Kunze, S., Schirrmacher, V., and Herold-Mende, C. (2004). Antitumor vaccination of patients with glioblastoma multiforme: a pilot study to assess feasibility, safety, and clinical benefit. *J Clin Oncol* 22, 4272–81.
- Steinman, R.M. and Banchereau, J. (2007). Taking dendritic cells into medicine. *Nature* 449, 419–26.
- Stuve, O., Marra, C.M., Bar-Or, A., Niino, M., Cravens, P.D., Cepok, S., Frohman, E.M., Phillips, J.T., Arendt, G., Jerome, K.R., Cook, L., Grand'Maison, F., Hemmer, B., Monson, N.L., and Racke, M.K. (2006). Altered CD4<sup>+</sup>/CD8<sup>+</sup> T-cell ratios in

- cerebrospinal fluid of natalizumab-treated patients with multiple sclerosis. *Arch Neurol* 63, 1383–7.
- Suter, T., Biollaz, G., Gatto, D., Bernasconi, L., Herren, T., Reith, W., and Fontana, A. (2003). The brain as an immune privileged site: dendritic cells of the central nervous system inhibit T cell activation. *Eur J Immunol* 33, 2998–3006.
- The Cancer Genome Atlas Research Network. (2008). *Nature* Sep 4. PMID: 18772890 [Epub ahead of print]
- Thomas, D.L., Kranz, D.M., and Roy, E.J. (2008). Experimental manipulations of afferent immune responses influence efferent immune responses to brain tumors. *Cancer Immunol Immunother* 57, 1323–33.
- Thurner, B., Roder, C., Dieckmann, D., Heuer, M., Kruse, M., Glaser, A., Keikavoussi, P., Kampgen, E., Bender, A., and Schuler, G. (1999). Generation of large numbers of fully mature and stable dendritic cells from leukapheresis products for clinical application. *J Immunol Methods* 223, 1–15.
- Toso, J.F., Gill, V.J., Hwu, P., Marincola, F.M., Restifo, N.P., Schwartzentruber, D.J., Sherry, R.M., Topalian, S.L., Yang, J.C., Stock, F., Freezer, L.J., Morton, K.E., Seipp, C., Haworth, L., Mavroukakis, S., White, D., MacDonald, S., Mao, J., Sznol, M., and Rosenberg, S.A. (2002). Phase I study of the intravenous administration of attenuated *Salmonella typhimurium* to patients with metastatic melanoma. *J Clin Oncol* 20, 142–52.
- Tsugawa, T., Kuwashima, N., Sato, H., Fellows-Mayle, W.K., Dusak, J.E., Okada, K., Papworth, G.D., Watkins, S.C., Gambotto, A., Yoshida, J., Pollack, I.F., and Okada, H. (2004). Sequential delivery of interferon-alpha gene and DCs to intracranial gliomas promotes an effective antitumor response. *Gene Ther* 11, 1551–8.
- Ueda, R., Low, K.L., Zhu, X., Fujita, M., Sasaki, K., Whiteside, T.L., Butterfield, L.H., and Okada, H. (2007). Spontaneous immune responses against glioma-associated antigens in a long term survivor with malignant glioma. *J Transl Med* 5, 68.
- Ulevitch, R.J. (2004). Therapeutics targeting the innate immune system. *Nat Rev Immunol* 4, 512–20.
- Van den Eynde, B., Peeters, O., De Backer, O., Gaugler, B., Lucas, S., and Boon, T. (1995). A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J Exp Med* 182, 689–98.
- Van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., De Plaen, E., Van den Eynde, B., Knuth, A., and Boon, T. (1991). A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 254, 1643–7.
- Van Meir, E.G. (1999). Cytokine expression in brain tumors: its role in tumor biology and tumor-associated immune responses. In *Inflammatory Cells and Mediators in CNS Diseases*. Ruffolo, R.R.Jr., Feuerstein, G.Z. Hunter, A.J. Poste, G. and Metcalf, B.W. (eds.), Harwood Academic Publishers, London. pp. 169–244.
- Walker, P.R., Calzascia, T., de Tribolet, N., and Dietrich, P.Y. (2003). T-cell immune responses in the brain and their relevance for cerebral malignancies. *Brain Res Brain Res Rev* 42, 97–122.
- Walker, P.R., Saas, P., and Dietrich, P.Y. (1997). Role of Fas ligand (CD95L) in immune escape: the tumor cell strikes back. *J Immunol* 158, 4521–4.
- Walter, L. and Albert, M.L. (2007). Cutting edge: cross-presented intracranial antigen primes CD8+ T cells. *J Immunol* 178, 6038–42.
- Wang, L.X., Huang, W.X., Graor, H., Cohen, P.A., Kim, J.A., Shu, S., and Plautz, G.E. (2004). Adoptive immunotherapy of cancer with polyclonal, 108-fold hyperexpanded, CD4+ and CD8+ T cells. *J Transl Med* 2, 41.
- Wang, L.X., Shu, S., Disis, M.L., and Plautz, G.E. (2007). Adoptive transfer of tumor-primed, in vitro-activated, CD4+ T effector cells (TEs) combined with CD8+ TEs provides intratumoral TE proliferation and synergistic antitumor response. *Blood* 109, 4865–76.
- Wang, L.X., Shu, S., and Plautz, G.E. (2005). Host lymphodepletion augments T cell adoptive immunotherapy through enhanced intratumoral proliferation of effector cells. *Cancer Res* 65, 9547–54.

- Wheeler, C.J., Black, K.L., Liu, G., Mazer, M., Zhang, X.X., Pepkowitz, S., Goldfinger, D., Ng, H., Irvin, D., and Yu, J.S. (2008). Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. *Cancer Res* 68, 5955–64.
- Wilmotte, R., Burkhardt, K., Kindler, V., Belkouch, M.C., Dussex, G., Tribollet, N., Walker, P.R., and Dietrich, P.Y. (2005). B7-homolog 1 expression by human glioma: a new mechanism of immune evasion. *Neuroreport* 16, 1081–5.
- Wintterle, S., Schreiner, B., Mitsdoerffer, M., Schneider, D., Chen, L., Meyermann, R., Weller, M., and Wiendl, H. (2003). Expression of the B7-related molecule B7-H1 by glioma cells: a potential mechanism of immune paralysis. *Cancer Res* 63, 7462–7.
- Wischhusen, J., Waschbisch, A., and Wiendl, H. (2007). Immune-refractory cancers and their little helpers—an extended role for immunotolerogenic MHC molecules HLA-G and HLA-E? *Semin Cancer Biol* 17, 459–68.
- Wood, G.W., Holladay, F.P., Turner, T., Wang, Y.Y., and Chiga, M. (2000). A pilot study of autologous cancer cell vaccination and cellular immunotherapy using anti-CD3 stimulated lymphocytes in patients with recurrent grade III/IV astrocytoma. *J Neurooncol* 48, 113–20.
- Wrzesinski, S.H., Wan, Y.Y., and Flavell, R.A. (2007). Transforming growth factor-beta and the immune response: implications for anticancer therapy. *Clin Cancer Res* 13, 5262–70.
- Wu, A., Oh, S., Gharagozlou, S., Vedi, R.N., Ericson, K., Low, W.C., Chen, W., and Ohlfest, J.R. (2007). In vivo vaccination with tumor cell lysate plus CpG oligodeoxynucleotides eradicates murine glioblastoma. *J Immunother* 30, 789–97.
- Wu, A.H., Xiao, J., Anker, L., Hall, W.A., Gregerson, D.S., Cavenee, W.K., Chen, W., and Low, W.C. (2006). Identification of EGFRvIII-derived CTL epitopes restricted by HLA A0201 for dendritic cell based immunotherapy of gliomas. *J Neurooncol* 76, 23–30.
- Yajima, N., Yamanaka, R., Mine, T., Tsuchiya, N., Homma, J., Sano, M., Kuramoto, T., Obata, Y., Komatsu, N., Arima, Y., Yamada, A., Shigemori, M., Itoh, K., and Tanaka, R. (2005). Immunologic evaluation of personalized peptide vaccination for patients with advanced malignant glioma. *Clin Cancer Res* 11, 5900–11.
- Yamanaka, R., Abe, T., Yajima, N., Tsuchiya, N., Homma, J., Kobayashi, T., Narita, M., Takahashi, M., and Tanaka, R. (2003a). Vaccination of recurrent glioma patients with tumour lysate-pulsed dendritic cells elicits immune responses: results of a clinical phase I/II trial. *Br J Cancer* 89, 1172–79.
- Yamanaka, R., Homma, J., Yajima, N., Tsuchiya, N., Sano, M., Kobayashi, T., Yoshida, S., Abe, T., Narita, M., Takahashi, M., and Tanaka, R. (2005). Clinical Evaluation of Dendritic Cell Vaccination for Patients with Recurrent Glioma: Results of a Clinical Phase I/II Trial. *Clin Cancer Res* 11, 4160–7.
- Yamanaka, R., Tsuchiya, N., Yajima, N., Honma, J., Hasegawa, H., Tanaka, R., Ramsey, J., Blaese, R.M., and Xanthopoulos, K.G. (2003b). Induction of an antitumor immunological response by an intratumoral injection of dendritic cells pulsed with genetically engineered Semliki Forest virus to produce interleukin-18 combined with the systemic administration of interleukin-12. *J Neurosurg* 99, 746–53.
- Yang, I., Kremen, T.J., Giovannone, A.J., Paik, E., Odesa, S.K., Prins, R.M., and Liau, L.M. (2004). Modulation of major histocompatibility complex Class I molecules and major histocompatibility complex-bound immunogenic peptides induced by interferon-alpha and interferon-gamma treatment of human glioblastoma multiforme. *J Neurosurg* 100, 310–9.
- Yu, J.S., Liu, G., Ying, H., Yong, W.H., Black, K.L., and Wheeler, C.J. (2004). Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. *Cancer Res* 64, 4973–79.
- Yu, J.S., Wheeler, C.J., Zeltzer, P.M., Ying, H., Finger, D.N., Lee, P.K., Yong, W.H., Incardona, F., Thompson, R.C., Riedinger, M.S., Zhang, W., Prins, R.M., and Black, K.L. (2001). Vaccination of malignant glioma patients with peptide-pulsed dendritic

- cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res* 61, 842–47.
- Zhang, J.G., Eguchi, J., Kruse, C.A., Gomez, G.G., Fakhrai, H., Schroter, S., Ma, W., Hoa, N., Minev, B., Delgado, C., Wepsic, H.T., Okada, H., and Jadus, M.R. (2007). Antigenic profiling of glioma cells to generate allogeneic vaccines or dendritic cell-based therapeutics. *Clin Cancer Res* 13, 566–75.
- Zhang, J.G., Kruse, C.A., Driggers, L., Hoa, N., Wisoff, J., Allen, J.C., Zagzag, D., Newcomb, E. W., and Jadus, M.R. (2008). Tumor antigen precursor protein profiles of adult and pediatric brain tumors identify potential targets for immunotherapy. *J Neurooncol* 88, 65–76.
- Zhu, X., Nishimura, F., Sasaki, K., Fujita, M., Dusak, J.E., Eguchi, J., Fellows-Mayle, W., Storkus, W.J., Walker, P.R., Salazar, A.M., and Okada, H. (2007). Toll like receptor-3 ligand poly-ICLC promotes the efficacy of peripheral vaccinations with tumor antigen-derived peptide epitopes in murine CNS tumor models. *J Transl Med* 5, 10.