Review article

Immunological aspects of intrinsic glial tumors

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Fundamental concepts of general tumor immunology and modes of immunotherapy are presented. Significant work dealing with the relationship of the immune system to intrinsic glial neoplasms is reviewed in relation to therapeutic applications and future investigative efforts.

**KEY WORDS** - tumor immunology - cerebral tumor - malignant glioma - tumor antigen - humoral immune response - cellular immune response - immunotherapy

A **ample** evidence exists for the presence of an immunological response that is initiated by antigenic components of various tumors. The perplexing therapeutic problem of intrinsic glial tumors has initiated interest in exploration of the relations of such lesions to the mechanisms of immunity. This paper will review fundamental concepts of general tumor immunology and present a compendium of significant work dealing with the relationship of the immune system to intrinsic glial neoplasms.

**Basic Elements of Tumor Immunology**

**Antigenicity of Tumors and Host Resistance**

Unlike grafts of normal tissues from syngeneic inbred animals, syngeneic or autologous tumors can give rise to a state of immunity in inbred tumor-bearing hosts. This has been demonstrated by surgical removal and challenge with the same tumor or injection of animals with radiation-attenuated tumor cells before challenge with viable cells. This immunity has been attributed to tumor-specific (or tumor-associated) transplantation antigens (TSTA) on malignant cells which are absent from normal tissues. These tumor antigens are often poorly immunogenic, meaning that the degree of immunity that is produced is relatively weak, perhaps sufficient only to cause the rejection of comparatively few cells.

Most experimentally induced tumors and certain apparently spontaneous neoplasms possess antigenic components that elicit immune rejection responses directed against the tumor. These antigens, like normal histocompatibility antigens, are located on the cell surface where they evoke immunological recognition and initiate the mechanism of rejection. The term "tumor-specific transplantation antigens" distinguishes these from other types of tumor-associated antigens that are also present on embryonic tissue and occasionally on normal adult tissues, but whose role in eliciting tumor rejection is less important.

The need for inbred animals has limited these studies largely to rodents, but in this experimental setting, various tumor types such as sarcomas, carcinomas, hepatomas, and lymphomas have now been investigated. The carcinogenic agents used in the induction of tumors include most of the well known chemical carcinogens as well as oncogenic viruses and physical agents such as inert plastic films, and ultraviolet and ionizing radiation.
Initial studies indicated a sharp division with regard to cross-resistance between tumors of viral origin and those induced by chemical or physical carcinogenic agents. Tumors induced by chemical carcinogens expressed individually distinct neoantigens, whereas the antigens on virally induced tumors were cross-reacting, that is, the specific transplantation antigens induced by DNA or RNA viruses were characteristic for all tumors induced by a particular virus regardless of the strain or even species of the host. However, it is now appreciated that chemically induced tumors express both individual characteristic tumor antigens and also cross-reacting antigens.

There is increasing evidence that at least some of the cross-reacting antigens are phase-specific or fetal antigens. Therefore, it is apparent that neoplastic cells may express several neoantigens such as: 1) tumour-specific antigens; 2) phase-specific antigens; 3) viral antigens; or 4) fetal antigens, depending on the tumor and the mode of induction. The exact mechanism for the origin of tumor-specific antigens is not clearly understood.

In man, the evidence for effective host reactivity against malignant disease is not as compelling as in rodents. There are, however, a small number of impressive examples from clinical investigations suggesting that under certain circumstances immunological reactions may influence malignant disease. Grace listed clinical observations suggesting the presence of a host's immunity to malignant lesions as: 1) spontaneous regression of tumors; 2) prolonged survival or cure of patients after incomplete removal of a malignant lesion; 3) sudden appearance of metastases many years after apparently successful therapy; and 4) regression of metastases after treatment of a primary lesion. Everson and Cole described 176 cases of proven spontaneous regression of malignant tumors in man. Further evidence for host immune control comes from the observation that immunosuppressive therapy or immunodeficiency diseases are associated with an increased incidence of malignancy. Doll and Kinlen reviewed over 4000 cases of renal transplants and reported 42 cases of malignant disease, an incidence of 0.42%. In the same article, they reported that among 200 cases with the diagnosis of ataxia telangiectasia, 14 patients developed malignant tumors (nine living with malignant lymphomas), and that among 90 cases of Wiskott-Aldrich syndrome, 11 patients developed primary malignant lymphoma. Both of these disorders are T-cell deficiency states with thymus aplasia. Thus, "immunosuppression," be it iatrogenic or due to a specific genetic abnormality, appears to play an important role in tumor development.

Immunosuppression, not only in the clinical setting but also in animal experiments, seems to be closely related to the onset of neoplastic disease. The issue is not so well defined that one can state that an immunological deficiency is a necessary prerequisite for developing a malignant neoplasm, for one does not need a grossly abnormal immunological system to develop cancer. A subtle defect, such as a transient immunosuppression or a very specific failure to recognize the tumor's antigens, may be involved. In many cases, however, the immunological deficiencies appear to result from the presence of malignancy rather than to precede it.

The hypothesis put forth to explain how the host defends the presence of tumors by reacting against tumor antigens is known as the concept of "immunological surveillance." Immunosurveillance by the delayed sensitivity reaction for the control of cancer was postulated by Greene. Burnet elaborated upon this concept, and suggested that cellular immunity acts as a major surveillance system against neoplastic mutations occurring in the body, and this continuously suppresses the growth of these cells. Experimental support for the surveillance theory derives from the fact that manipulations known to inhibit the immune response, such as total body radiation, neonatal thymectomy, or treatment with antilymphocyte serum, increase the incidence and hasten the appearance of certain tumors. While the original views of the role of T-cells as the surveillants of tumor-associated antigens now seem too simplistic and, indeed, the whole concept of surveillance has been seriously questioned, surveillance remains an attractive testable hypothesis from which to approach the host-tumor relationship.

Components and Regulators of the Immune Response

The immune response against tumor-specific antigens may be mediated independently or in concert by three cellular components of the immune system: T-lymphocytes, macrophages, and B-lymphocytes.

Cellular Immune Response. One major type of immune response is termed "the cell-mediated immune response." The activation of this response results in the production of sensitized lymphocytes and macrophages that are capable of destroying tumor cells. Perhaps the most important cell of the cell-mediated vanguard is the thymus-derived lymphocyte or T-cell. These are lymphocytes that originate as stem cells in the bone marrow and migrate to the thymic cortex where they proliferate and are influenced by thymic hormones to become T-cells. They reside in all lymphatic organs of the body. When stimulated by anti-
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gen, these cells undergo prompt division and release into the circulation.

The macrophage, a second major cellular protagonist, is of prime importance in processing antigen and presenting it to immunocompetent T-lymphocytes, therefore initiating T-cell activation. Following the recognition of antigens and proliferation of activated cellular elements, the T-cell enters its functional phase, the effector phase, during which it is capable of damaging or destroying tumor cells. This is accomplished by direct interaction or through the release of cytotoxic substances, such as lymphotoxins, which can lyse tumor cell membranes. However, much of the effectiveness of T-cells depends on soluble mediators (lymphokines) which they release.

There are several subclasses of T-lymphocytes; each subclass possesses a characteristic phenotype and function. In mice, at least three subclasses of T-lymphocytes have been recognized: the cytotoxic (killer) effector cell; the helper T-cell, which aids in antibody production; and the suppressor T-cell, which regulates the immune response to antigens, including tumor antigens. Suppressor T-cells deserve particular mention. These critical cells are capable of suppressing the rejection of tumors in inbred mice that have been immunized with the specific tumor antigen. They arise within 24 hours of antigen exposure and are found predominantly in the thymus and spleen of the host. These cells appear to limit the cytolytic tumor response of the host. Furthermore, the immune regulatory activity of the suppressor T-cell is highly specific and antigen-specific. Suppressor T-cells and the antigen-specific suppressor factors they release may interfere with the development of helper and/or effector T-cells in the tumor-bearing host.

"Natural killer" (NK) cells have also been described and have been alleged to be of importance in the defense against the development of tumor cells into full-fledged neoplasms. Natural killer cells may be a variety of T-cells or perhaps immature mononuclear cells. These cells can lyse a variety of tumor cells in vitro without prior sensitization, and do not require antibody. Their exact significance in vivo even in the mouse is uncertain, but work on NK cells is intense at the moment and should soon clarify the situation.

The macrophage also appears to rise from progenitor cells in the bone marrow via circulating monocytes. Macrophages are involved in almost every phase of the immune response. Not only are they important as effector cells, but there is now a wealth of information suggesting that macrophages may, through a combination of suppressing and enhancing effects, play an important role in regulating the immune response. The macrophage acts as a processing cell for antigen, and collaborates with T-cells in tumor destruction. There is also abundant evidence that presentation of antigens to T-cells, including tumor antigens, by the macrophages is an essential part of the primary response. Macrophages also make lymphocyte-activating factor (LAF), which causes proliferation of T-cells. Effector macrophages are initially attracted to an antigenic target by chemotactic factor from a T-cell, and then immobilized in its vicinity by macrophage-inhibitory factor (MIF). Finally, it is activated by macrophage-activating factor (MAF), which is probably the same as MIF, to become a cytotoxic effector cell. Macrophages appear to be a functionally heterogeneous group. At the present time it is not clear whether heterogeneity is simply the result of different levels of maturation or whether there are, in fact, phenotypically distinct populations, as with T-cells. To continue the parallel with T-cells, there are suppressor macrophages. These suppressor cells have a profound effect on the activity of T-cells. Although a number of reports describing suppressor effects of macrophages imply that cell-to-cell contact is required, macrophages may well mediate suppressor effects also through soluble factors, of which prosta
glandins have recently attracted considerable interest.

Humoral Immune Response.

The second major component of the immune response is termed the "humoral immune response:" the formation of antibodies to specific antigens by B-lymphocytes. This cell also originates in the bone marrow and is influenced to become a competent cell elsewhere, perhaps in the lymphoid tissue in the wall of the gastrointestinal tract. Upon stimulation by antigens, B-cells proliferate and differentiate into plasma cells, which actually secrete the antibodies. Even in the presence of adequate numbers of antibody-producing cells, antibody production is dependent upon sufficient numbers of helper T-cells.

Circulating antibodies reacting with antigens are detectable in many tumor systems. These immunoglobulins (Ig) are principally IgG and IgM. IgM antibodies in the presence of complement may be cytotoxic in vitro to tumor cells. IgG antibodies may aid in cell-mediated killing by arming such cells as macrophages to recognize and ultimately destroy the tumor target. These are "cytphilic antibodies." The killing, which appears to be independent of complement, is often called "antibody-dependent cell cytotoxicity" (ADCC), particularly if the effector cells are not known to be macrophages. Antitumor antibody is of secondary importance in the defense against solid tumors, but can be useful particularly in arming against dispersed tumors such as leukemia. They are
also of considerable utility to immunologists in the study of tumor antigens.

**Blocking Factors and Escape Mechanisms**

Even though lymphocytes from tumor-bearing animals can often kill cells from the respective tumors *in vitro*, neoplasms that possess tumor-specific transplantation antigens (TSTA) are usually not rejected by the hosts *in vivo*. Rather, for tumors carrying TSTA to be rejected, the animals must first be immunized against the tumor and the cell dose used for subsequent challenge must be greater than one to three orders of magnitude above the minimal dose used for outgrowth in unimmunized controls.

Several mechanisms that contribute to the escape of antigenic tumors from immunological controls have been described. The concept of blocking factors was originally introduced *in vitro* by the Hellstroms. These specific blocking factors that inhibit the *in vitro* destruction of tumor cells by immune T-lymphocytes represent one of the more extensively studied immunologically specific escape mechanisms. Initially, it was considered that blocking was due to antibody. However, Sjögren, *et al.*, suggested that the blocking factor was probably an antigen-antibody complex. Antigen-antibody complexes can induce suppressor T-cells, and the latter probably cause enhanced tumor growth. More recently, it has become apparent that blocking factors may activate suppressor T-cell subsets *in vitro* by the Hellstroms. These specific blocking factors that inhibit the in *vitro* destruction of tumor cells by immune T-lymphocytes represent one of the more extensively studied immunologically specific escape mechanisms. Initially, it was considered that blocking was due to antibody. However, Sjögren, *et al.*, suggested that the blocking factor was probably an antigen-antibody complex. Antigen-antibody complexes can induce suppressor T-cells, and the latter probably cause enhanced tumor growth.

More recently, it has become apparent that blocking factors may activate suppressor T-cell subsets to elaborate glycoproteins, which are the active agents that interfere with effector cell subsets. Concurrently, suppressor macrophages also elaborate mediators, namely prostaglandins, which impair the development of effector cells too, principally by inhibiting the proliferation of T-cell precursors.

Another potential mechanism of blockade is one in which a soluble antigen may block cytotoxic T-lymphocytes via interaction with specific receptors. This specific alteration may effect either the expression of already sensitized T-cells or may prevent precursors of antigen-sensitive cells from turning into immune cytotoxic cells, thereby developing tolerance in the host to the antigen in question.

**Immunotherapy**

The goal in studying relationships of the immune system to neoplasia is to capitalize on the response and to successfully treat the tumor-bearing patient. In this regard, the relationship between effector mechanisms of the immune system and blocking factors is critical. The effectiveness of the immune system in reducing tumor burden may be compromised by the presence of soluble tumor antigen, antigen-antibody complexes, and nonspecific suppressor factors generated by the tumor that are circulating in the serum. At the same time, suppressor subsets within the T-cells and macrophages directed largely by these humoral components may effect a depression of cellular effector mechanisms. Therefore, the prime objectives of immunotherapy are: 1) activation of antitumor cell-mediated cytotoxic responses; 2) activation of humoral cytotoxic responses; and 3) mitigation of blocking factors and cellular suppressor responses.

Both laboratory and clinical data have shown that if a tumor mass is small, immunotherapy alone may be effective. Antigenic tumor autografts of $10^4$ or fewer cells are often rejected in mice, particularly with the aid of immunotherapy; whereas autografts of greater than $10^8$ cells are uniformly successful in defeating efforts of the immune system to eradicate tumor burden. Since $1\text{ cu cm}$ of tumor is approximately $10^9$ cells, a corollary is that cytoreductive therapy may first be needed to reduce the critical neoplastic mass.

It has been demonstrated experimentally that immunotherapy with immunostimulants can occasionally promote or enhance tumor growth. The major factor involved may be related to stimulation of suppressor cells. Both neoplasms and many conventional antitumor therapies have the potential for inhibiting the immune capacity of the host, and some investigators have stated that immunotherapy should not be attempted in immunologically impaired patients. However, some forms of immunotherapy can restore immunity toward normal and others transfer immunity to impaired hosts. Certain immunomodulators can stimulate immunity, antagonizing suppression as long as some competence remains.

Five major modes of alteration of the immune response are now employed in the treatment of neoplastic diseases. These we have termed “active non-specific,” “active specific,” “adoptive,” “passive,” and “immunorestorative.” These modes of therapy are initiated by agents that have been termed “biological response modifiers.”

Active nonspecific immunotherapy employs compounds or materials that do not necessarily have an antigenic similarity to the tumor, but increase the general immune capacity of the host. These include a variety of substances such as the microorganisms *Corynebacterium parvum* and *Corynebacterium parvum* calmette-guérin (BCG) and as chemical agents such as pyran and fluorenone derivatives, and interferon inducers such as polyadenylic-uridilic acid (poly AU) and polynosinic-cytopylic acid (poly IC). In experimental systems, these agents have the capability of preventing tumor growth and aborting the growth of small established tumors. These agents generally boost both cell-medi-
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ated and humoral immune responses and are capable of reversing to a certain extent the immunosuppressive effects of antitumor agents. However, they may increase negative influences upon immunity too, including suppressor T-cells and suppressor macrophages, and perhaps the antibody component of blocking factors as well. In addition, most of these agents have a number of significant systemic and local toxic effects. Cell wall fractions of microorganisms, particularly BCG and C. parvum have been prepared in an effort to circumvent the toxicity of the whole organisms. These are, in general, either lipid-free extracts (cell wall skeletons) or defined small lipid materials, such as the "P3" (cord factor), a dimycolate of trehalose from BCG.

Active specific immunotherapy utilizes substances that are antigenically related to products of the tumor. A specific antigen, such as tumor cells from the individual patient or another patient with an antigenically similar tumor, may be employed. It is known that the antigenicity of tumor cells may be increased by removing the sialic acid coating around the cells with neuraminidase. Tumor cells that have been killed with either x-ray or chemical agents such as mitomycin-C have been used to prepare cell-free antigen extracts. These have been utilized to augment the immune response in general; however, it is apparent that the use of soluble antigen extracts should be approached with caution because excessive amounts might lead to blocking of the cell-mediated response.

It is logical, and a subject of current study, that combined specific and nonspecific active immunotherapy may be undertaken in which immunomodulators may be used in conjunction with specific tumor extracts, preferably as a vaccine containing both components. The immunomodulators would then be true adjuvants to the specific immunogens, tumor antigens, which seems to be their most appropriate use.

Adoptive immunotherapy (transferring immunity with lymphoid cells or subcellular information) was initially attempted by the transfer of histocompatible lymphocytes from one patient to another. These care-fully matched lymphocytes can prevent tumor growth in experimental systems. In addition, lymphocytes may be transferred from the peripheral blood to tumor.

Recently, interest has been concentrated on the concept of transfer of antitumoral immunity at the informational level by utilizing components extracted from sensitized lymphoid cells. These substances in the past have included immune RNA, which is a phenol extract of lymphocytes prepared in laboratory animals, and transfer factor, which is a cell-free extract of lymphocytes with a molecular weight of less than 10,000. Most recently, considerable interest has been generated by the interferons, a family of inducible secretory glycoproteins now designated α, β, and γ, produced both in vivo and in vitro by eukaryotic cells in response to viral infections or other stimuli. They appear to be intercellular messengers able to adoptively transfer immunological messages; upon binding to specific cell receptors, they can direct cells to alter the expression of some of their specialized functions. Interferons have, therefore, been aptly termed "inducible inducers." In addition to antiviral effect, interferons have a profound direct effect on cell growth and appear to confer a degree of protection against aberrant cells, including neoplastic cells. Interferon-γ (immune interferon) is a soluble mediator produced by sensitized T-cells. Interferons are also immunomodulators. Although they appear to depress lymphocyte proliferation in response to mitogens, they augment the specific cytotoxic responses of sensitized lymphocytes two- to fivefold. In addition, human antibody-dependent cell-mediated cytotoxic responses are significantly augmented, as is the phagocytic activity of macrophages both in vivo and in vitro. The cytotoxicity of macrophages and NK cells against their respective target cells is also significantly aug-

Immunorestitution of depressed cell-mediated immunity may be undertaken with agents such as levamisole, which has a particular impact on cell-mediated aspects of immunity. Another class of substances that are currently under evaluation are the thymic hormones, of which a number of fractions have been isolated. These compounds have a particular impact on the T-cell subsets, and have the capability of restoring depressed T-cell populations to normal levels, both from the quantitative and qualitative physiological levels of evaluation. Other techniques that are being investigated to restore cellular components of the immune response include the utilization of Cytoxan to inhibit suppressor cells, and antiprostaglandins, which block the immunosuppressive action of these agents.

Passive immunotherapy by administration of antitumor antibodies, particularly monoclonal antibodies that have been produced by hybridoma technology, is another potential avenue for reduction of tumor burden. This form of treatment has been effective in rodents against dispersed tumors, particularly leukemia. It carries the inherent danger that blocking factors (antigen-antibody complexes) may be created in vivo, with a consequent enhancement of tumor growth. Antibody-dependent cell-mediated killing of tumor cells is a likely mechanism by which highly specific antitumor monoclonal antibodies might aid
the host. Furthermore, antibody conjugated to cytotoxic chemicals, biological molecules, or radioisotopes may be able to direct therapy more precisely to the sites of tumors. The potential importance of immunotherapy with antibodies justifies further clinical exploration, despite any theoretical objections.

**Immunology of Intrinsic Glial Neoplasms**

**Immunological Privilege**

A foreign skin graft transplanted from one individual to another will be rejected unless the donor and recipient are identical twins. Medawar found that if a fragment of foreign skin was implanted into the brain of a rabbit, it was not rejected. He concluded that the rejection process could not act in the brain, which was "an immunologically privileged site," meaning that antigens within the brain do not evoke an afferent response, probably by reason of lack of lymphatic drainage and effective maintenance of the blood-brain barrier. Medawar's statements were influenced by the work of Murphy and Sturm, who demonstrated that tumors transplanted to the brain would often grow, although the same tumor transplanted subcutaneously was rejected. Subsequently, the work of Greene and others appeared to substantiate Medawar's conclusions.

A substantial body of data has emerged which effectively refutes this concept. Neoplastic processes within the axial neuronal components demonstrate permeability of the blood-brain-tumor barrier during radioisotopic brain scanning procedures and electron microscopic examination. Glioma tumor cells have been detected in the venous channels of patients undergoing craniotomy.

Scheinberg, et al., showed that chemically induced ependymoblastomas obtained from inbred mice could be successfully transplanted intracerebrally 100% of the time into other animals of the same strain but only 10% to 35% of the time into allogeneic mice. Cure of ependymoblastomas by radiation resulted in rejection of subsequent tumor challenges into either the brain or skin. Similarly, radiation cure of subcutaneous tumor rendered a significant number of animals refractory to subsequent intracerebral or subcutaneous tumor implants. Prior immunization of mice with tumor cells incorporated in complete Freund's adjuvant resulted in inhibition of growth in 85% of subsequent implants. These studies have been confirmed by others, and offer a clear demonstration of incomplete immunological privilege.

It is apparent that effector cells of systemic origin can enter the brain. The initial observation by Ridley and Cavanagh that more than 50% of malignant gliomas show characteristic infiltration of lymphocytes and monocytes in perivascular and diffuse sections of the tumor has been confirmed by others. In addition, experimental allergic encephalomyelitis (EAE) can be induced directly or by passive transfer of cells.

Therefore, it is apparent that the concept of the brain as an immunologically privileged site is unwarranted and that the premise of "partial" privilege is more appropriate in line with evidence which has pointed toward the occurrence of: 1) intracerebral graft rejection; 2) induction of a systemic immune response secondary to intracerebral implants; and 3) entry of effectors of systemic origin through brain parenchyma. It would appear that loss of privilege is determined by alteration in barrier integrity which occurs as a result of structural changes inherent in neoplastic vasculature, trauma, or as occurs in reactive inflammatory processes, such as EAE.

**Organ- and Tumor-Related Antigenic Components**

**Normal Brain Antigens.** Investigative effort has disclosed a number of normal brain and nervous system antigens, many of which are evident throughout the vertebrate fila. Those of intracellular location have been most extensively defined and include S-100 protein, 14-3-2 protein, glial fibrillary acidic protein (GFA), alpha-2 glycoprotein, and myelin basic protein (MBP). Cell-surface antigens have been reported in normal brain; however, strict biochemical definition of these components has not been realized. The study and definition of normal glial-specific surface antigens has been complicated by the presence of antigenic components that are shared with microorganisms or other vertebrate tissues. In particular, complement-fixing IgM antibodies that are highly reactive with normal brain have been described in patients infected with *Mycoplasma pneumoniae*, and a large number of reported interspecies brain-associated antigens are also expressed on lymphoid cells. Human fetal glial-specific antigens have been reported.

"Shared" Brain and Tumor Antigens. Initial studies relating to neural tumor antigenicity were undertaken over 40 years ago. In 1936, Siris, employing rabbit antiserum that had been prepared against aqueous extracts of normal brain or glioblastoma tissue, demonstrated similar reactivity profiles in complement fixation assays. Following this, numerous investigators have substantiated the inferences of these data, namely, that glial tumors share certain elements of their antigenic substrate with normal brain. Although organ-specific antigens are readily identifiable in tumors of the glioma spectrum,
there appears to be a reduction in the expression of these components with advancing stages of malignant transformation.\textsuperscript{133} This trend is demonstrated in the work of Wickremesinghe and Yates,\textsuperscript{137} who postulated that the loss of organ-specific antigens from glioblastoma multiforme tissue that they observed is part of a continuum. The glial-specific antigenic component is present in the benign and less anaplastic cells and largely absent from highly anaplastic cells, representing qualitative and quantitative differences between normal and neoplastic cells in the distribution of antigens in cell membranes.\textsuperscript{138} Concurrently, this quantitative and qualitative associated antigenic variability of neoplastic glial cells has been stressed by Wikstrand, \textit{et al.}\textsuperscript{140} who showed that normal brain antigens expressed in human gliomas are quite immunogenic. Nonhuman primate antisera raised against glioblastoma multiforme tissue or cultured cell lines were used to demonstrate unique patterns of normal adult and fetal brain-associated antigenic expression by a large panel of cultured cell lines derived from human glioblastoma multiforme. Although the association of antigen expression and degree of anaplasia was observed in these studies, the highly variable expression of normal brain-associated antigens was postulated to reflect the unique characteristics of the cell population that gave rise to these tumors. Although certain contradictory studies exist, this trend appears to be maintained in most studies that have dealt with identification of S-100 protein, GFA protein, and alpha-2 glycoprotein in tumors of the glioma series.\textsuperscript{24,26,136} Regarding this well characterized antigenic component, there appears to be concurrent evidence that normal human GFA and that isolated from glioblastoma tissue are immunologically identical,\textsuperscript{22} and that non-astrocytic tumors in general have no or very little GFA.\textsuperscript{56} At the same time, GFA concentration is proportional to the number of astrocytes in the tumor, and decreases with increasing malignancy.\textsuperscript{56,134}

\textbf{Study of Glioma Antigens Through the Preparation of Hetero-Antisera.} Interspecies preparation of antisera to glioma cells is fraught with difficulties related to specificity of response. These technical difficulties were observed in certain studies\textsuperscript{76,103} in which rabbit antisera were prepared to the human glioma cells. Absorption studies were then demonstrated to be incomplete, and significant cross-reactivity following brain absorption was apparent.

Utilizing lyophilized glioblastoma tissue, Wahlström, \textit{et al.},\textsuperscript{138} prepared rabbit antisera. Following multiple injections with glioblastoma tissue, the animals developed EAE, after which the serum was collected, and absorption tests were performed with a battery of normal tissues, including brain, until they reacted only with cell lines cultured from malignant gliomas by indirect immunofluorescent assays. In a coded series of 23 cell lines derived from different normal and neoplastic tissues, all of the 15 lines included were correctly identified by what was considered to be “specific” membrane antigens. However, the possibility of cell line contamination by \textit{Mycoplasma} was not considered.

Mahaley\textsuperscript{60} prepared antiglioma antibodies from human glioblastoma tissue. Following extensive absorption and elution experiments, he conjugated the globulins with \textsuperscript{125}I. Three to 5 days before reexploration, the patients had intra-arterial injections of 50 \(\mu\text{g}\) of the preparation. At surgery, samples were collected from the tumor, adjacent gliotic regions, and normal brain. Sixteen of 18 patients demonstrated concentrations of antibody within the tumor that were higher than those in either gliotic tissue or normal brain. Concurrently, control proteins conjugated with \textsuperscript{131}I and Cytoxan (cyclophosphamide) \textsuperscript{14}C were injected with unit concentrations within the tumor versus normal brain, being observed to be lower than the antibody to normal brain ratios in eight of 10 cases. In spite of these apparently encouraging findings, unit concentrations of antibody within tumor tissue were not sufficient to warrant pursuing the investigation.

Coakham and Lakshmi\textsuperscript{18} raised a rabbit antiserum to one of seven cultured human astrocytomas and assayed its antibody activity on the original tumor cells by dye exclusion cytotoxicity testing. After repeated absorption with brain and other tissues, a constant cytotoxic effect remained, indicating tumor-associated antigens. In a series of absorption experiments, astrocytoma-associated antigen was detected in six of seven astrocytoma cultures, but was not found in a series of other human tumors and tissues tested. Homogenates of two original astrocytomas absorbed out associated antigen activity, therefore implying the presence of an antigen \textit{in vivo}. However, the serum was not definitely HLA-nonreactive, nor were the cultures shown to be \textit{Mycoplasma}-free.

Wikstrand, \textit{et al.},\textsuperscript{140} studied surface antigenic characteristics of human glial brain tumors using a complement-dependent cytotoxic antibody assay and indirect membrane fluorescence. Eight permanent, well characterized cell lines derived from human gliomas were utilized for analysis, with sera raised by hyperimmunization of nonhuman primate with glioblastoma multiforme tissue or established human glial brain tumor lines. Extensive absorption of nonhuman primate and antiglioma sera removed all activity for a series of nongliomatous cell lines tested, but left
significant activity against a glioma tumor cell line-associated antigen present in all eight glioma lines tested. In a continuation of the latter experiment, Wikstrand and Bigner demonstrated that three antisera, rendered specific for human glial brain tumors, contained antibodies directed against normal adult and fetal human brain-associated antigens by indirect absorption analysis and direct cytotoxic antibody testing. Their studies indicated that the normal adult and fetal glial-associated antigens detected by these studies were not cross-reactive, interspecies antigens or shared brain lymphoid cell antigens, but antigens which are expressed on normal adult brain, fetal brain, and cell lines derived from human glial brain tumors.

Kehayov immunized rabbits with saline extracts of either normal brain or human glioblastoma in complete Freund's adjuvant followed by multiple immunizations without adjuvant. Sera obtained after this long-term immunization were analytically absorbed and assayed by immunodiffusion and immunoelectrophoresis versus saline extracts of adult and fetal brain, non-nervous system organs, and a large panel of gliomatous and nongliomatous tumors. Antiglioblastoma sera after absorption with human organ extracts, including normal adult brain, gave one precipitin line in immunodiffusion with 13 of 16 glioblastomas and nine of nine astrocytomas. The sera gave no reaction with normal adult brain, meningiomas, neurinomas, or cerebral metastasis of non-nervous system origin. Adult brain-absorbed antisera did react with extracts of human fetal brain of 8 to 10 weeks' gestation. However, one of the apparent tumor-specific antigens recognized by the antiglioblastoma serum migrated to the beta zone and the second to the alpha-2 zone. The 8-week fetal brain-associated antigen migrated separately.

To date, studies related to hetero-antisera preparation have failed to produce evidence suggestive of precise antigenicity related to these tumors. The paramount issue is “specificity” of the observed responses, and it is evident that considerable care is needed in the experimental sector before an absolute statement can be made to resolve this issue. Exhaustive absorption studies to exclude nonspecific antigenic determinants as well as care related to contamination of culture substrates with microbial organisms are necessary before specificity may be implied. The foregoing studies have suggested the presence not only of shared antigenic components with normal brain and fetal tissues, but also with other tumor systems as well. One of the major questions that remains unanswered with these investigative efforts relates to the origin of the antigenicity of these tumors. Do the antigens represent virally coded antigens, fetal antigens which are reexpressed by derepression of genetic information, or are they antigenic components that have arisen de novo in transformation? There has been no consistent demonstration of virally related antigenic constituents associated with malignant glial tumors, and, although some controversy exists, it does appear that at least some glial tumors share antigenic constituents with fetal tissues.

Recently, approaches to the serological definition of cell surface molecules by hetero-immune sera have been revolutionized by the introduction of hybridoma methodology. There is considerable hope that monoclonal antibody produced by the hybrid progeny of a myeloma cell and a normal immunoglobulin-secreting B cell will replace the need for conventionally prepared allo-antisera and hetero-antisera. Although a number of technical problems need to be resolved, application of the methodology in its present form has already resulted in the availability of monoclonal antibodies to histocompatibility and differentiation antigens on mouse, rat, and human cells. With the intense interest and activity in this area, we may expect that monoclonal antibodies against tumorspecific cell surface antigens of human cancers will also be produced.

Therefore, on the basis of studies related to the preparation of hetero-antisera, it may be stated that no specific glioma-related antigen has been defined. The question of whether specific antigens exist is controversial as it is with most other malignant neoplasms. Consideration of humoral and cellular responses in glioma-bearing patients may shed further light on these issues.

Humoral Immune Responses in Human Gliomas

Since it is apparent that an element of mixed antigenicity is inherent in intrinsic glial neoplasms, the next consideration relates to the capability of these tumors to elicit an immune response in the host. Numerous complex factors bear on this issue, including the sensitivity and capability of assays that are employed to detect the presence of such antibodies. Parallel consideration of issues of antigenicity and humoral responses are appropriate because of the fact that humoral immune responses are effective screens for the definition of antigenic constituents in any given tissue.

Early studies suggested the presence in the blood of tumor-bearing patients of circulating humoral components that could modify the biological activities of glioma cells in vitro. However, initial efforts to detect circulating antibodies in patients harboring such tumors by an indirect membrane fluorescence technique rendered little evidence of such a re-
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Lewis, et al.,63 introduced the concept of sequential expression of various membrane and cytoplasmic antigenic components during the course of the tumor's natural history. This gave impetus to studies employing an indirect immunofluorescence technique on a selected battery of cytoplasmic glioma cell substrates which demonstrated the presence of circulating antibodies to the cytoplasmic contents of these tumor cells in a heterologous system in 50% of glial tumor-bearing cases and only 7% of tumor-bearing or normal controls.111 No reactions were observed in incubations with normal brain tissues, and absorption studies indicated the presence of a tumor-associated antigen. Other investigators have disclosed evidence of circulating antibodies related to tumor cells by this technique.17,117 However, because of the incidence of positive response in relation to other tissue types and normal controls, no claim regarding specificity of response can be justified. Such a suggestion of shared antigenicity is also apparent in microimmunodiffusion studies141 in which soluble antigens from human meningiomas were used to screen brain-tumor patients for antibodies. The sera of 63% of meningioma patients, 53% of glioma patients, and 17% of other tumor patients yielded positive responses. Pfreundschuh, et al.,96 conducted an extensive serological analysis on 30 patients with astrocytomas. These sera were tested for antibody reacting with cell-surface antigens of cultures of autologous astrocytoma cells. Absorption analysis of reactive sera with autologous, allogeneic, and xenogenic cells permitted the definition of three classes of astrocytoma cell-surface antigens: Class I antigens, showing absolute restrictions to autologous astrocytoma cells; Class II antigens, showing identification in all astrocytomas tested as well as on neuroblastoma, sarcoma, and some melanoma lines (these antigens were not found on cell lines derived from carcinomas or normal tissues); and Class III antigens, widely distributed on cultured normal and malignant cells of both human and animal origin.

The previous studies, although indicating the presence of circulating antibody, gave no evidence related to the function of these immunoglobulins. Kornblith, et al.,54 applied an in vitro microcytotoxicity assay to study the immune responses in individuals with astrocytomas. Designing a complex and rigorously controlled system, they demonstrated evidence of significant cytotoxicity in 65% of sera tested against allogeneic astrocytoma cells. Over a 12-year period this assay was refined from the standpoint of its components, and in a recent report55 this group demonstrated evidence of significant cytotoxicity in 82% of sera taken from astrocytoma cases. In general, positive results were more frequently obtained in lower-grade astrocytomas. However, meningiomas, acoustic neuromas, pituitary adenomas, and metastatic tumors showed elements of positivity in a variable number of cases. This observation implied that the detection of antibody was not related to a tumor-associated antigen, but that the assay detected antigens that were shared by tumors originating from the neuroectodermal germ layer. These findings are in agreement with others,50,94 and it is apparent that, within the cytoplasm and surface component of glial neoplasms, proteins are present that have the character of elemental brain protein, tumor-associated components, and possibly specific tumor antigen.

Although it has been postulated that certain disease processes may initiate the local formation of antibody within the neural axis,106 most investigators would consider that immunoglobulins of systemic origin have access to the neural tissues via altered capillary walls. Relative to this issue, Tabuchi and Kirsch121 demonstrated the presence of IgG on tumor cells of three of nine tested glioblastoma multiforme specimens by an immunoperoxidase technique, and considered that this was presumptive evidence for the presence of antigen-antibody binding in vivo. The investigations of Aarli, et al.,4 have shown that normal human IgG binds to neurons, glia, and myelin sheaths; therefore, it is difficult to presume specificity of response in these studies.

From the foregoing data, it is apparent that few investigations, in the light of rigorous criticism related to assay technique and experimental design, present credible data for the presence of specific glioma antigens. Certainly, no evidence for specific humoral immunity has been presented. Future studies require refinement of assay systems and strict observation of control parameters in order to provide credibility with regard to the immune response and the presence of antigenic components related to these tumors.

Cellular Immune Responses and Altered Immune Status

Evidence of Cellular Immune Responses. Round-cell infiltrations, particularly in the perivascular regions, may be a characteristic component of the histological features of malignant gliomas. Following the initial report by Ridley and Cavanagh102 of an overall 65% incidence of cellular infiltrations in autopsy material, a number of other investigators have undertaken studies in an effort to determine the incidence and significance of these cellular infiltrations,10,91,120 Studies by Morantz, et al.,79,80 in both laboratory and clinical settings have disclosed that a significant number of the cellular components represent macrophages.
T-cells likewise comprise a major subpopulation of the infiltrating round-cell groups. It is apparent that in some intrinsic neural tumors the primary cytotoxic cellular component of the immune system has ample representation within the cellular matrix of the neoplasm. Independent studies by Brooks, et al., and Palma, et al., have indicated that individuals who show significant infiltration of round cells within their tumors have longer survival courses than those who show no evidence of such response on surgical biopsy material.

However, the data of Albright, et al., have indicated that an intracerebral response in glioma patients is likely to be inadequate. In a study designed to test the potential of intratumoral inoculation of purified protein derivative (PPD) in BCG-immunized patients to induce a localized immune response, these authors demonstrated that none of five patients intracerebrally inoculated developed a degree of inflammation that was more than moderate, or an inflammatory infiltrate capable of encompassing the periphery of the tumor. This relative lack of response occurred in all patients regardless of the degree of the response to intradermal PPD.

Coincident with anatomical evidence regarding the presence of small cells within the tumor matrices, a number of issues regarding cellular immunity deserve further scrutiny. These include: 1) sensitization of peripheral leukocyte subgroups to tumor-associated antigenic components; 2) physiological effectiveness of these cellular components within the host; and 3) general cellular immune competence.

As in studies of humoral immunity, a major limiting factor related to the reliable detection of specific cell-mediated immunity to glioma antigens has been the lack of an identifiable specific antigenic substrate for use in in vitro analysis or in vivo studies of patient reactivity. Certain studies that have claimed to show specific reactivity have failed to characterize the biochemical nature of the antigenic substrates that have been used to demonstrate evidence of specific cell-mediated immunity.

Sheikh, et al., addressed the question of sensitization of leukocyte subgroups in patients bearing malignant glial tumors by using the leukocyte adherence inhibition assay. Eighty percent of glioma-bearing patients demonstrated significant inhibition of leukocyte adherence in the presence of uncharacterized 3M KCl extracts of glioma tissue as compared to normal brain extracts. Appropriate controls indicated that this response was gliatumor-associated, and defined a sensitization of leukocytes to gliomatous antigenic substrates.

Assays devised to demonstrate cytotoxic capabilities are subject to complications in variables and controls. Early poorly controlled experiments suggested cytotoxic capability of lymphocytes exists in glioma-bearing cases. In addition, in vivo autologous implant experiments implied that cell-mediated immune rejection could be elicited by malignant gliomas. However, Hitchcock, et al., in the only study in which antigen preparations used for in vivo testing were analyzed by polyacrylamide gel electrophoresis, showed that the "glioma-specific" antigenically active fractions in tests of cutaneous delayed hypersensitivity contained antigenic components shared with normal white matter, thereby contradicting a premise of a specific response.

In later, more detailed, and better controlled studies, Levy investigated the specificity of lymphocyte-mediated in vitro cytotoxic reactions in a series of patients with primary intracranial tumors. Specific tumor-directed lymphocyte cytotoxicity was observed in 35 of 41 patients with glial tumors. Tumor specificity of the reactions was affirmed by testing lymphocytes of each patient and controls against nine to 12 different target cells. The target cells were each from an early passage in culture and included both neoplastic and normal cells from a given patient as well as allogeneic tumor cells of types both related and unrelated to the patient's tumor. Specificity was further affirmed by showing that cytotoxic responses could be abolished by prior absorption of the lymphocytes on appropriate cell monolayers. In addition, direct assays in concert with the absorption studies showed that, in glioma patients, the cytotoxic response was directed against two different antigenic determinants. One was found on cells from all glial tumors regardless of the degree of anaplasia: a common glioma antigen. The other was expressed on anaplastic gliomas, melanomas, and fetal cells, but not on well differentiated gliomas, normal adult glial cells, fetal fibroblasts, or other tumors. Distribution of the second antigen was believed due to the common origin of melanocytes and glial cells from the ectodermal cells of the embryonic tube.

However, Woosley, et al., in a meticulous study of cytotoxicity using the same assay system, demonstrated a significant cytotoxic response to patient effector cells in a glioblastoma in only seven out of 36 cases. The same group also investigated antibody-dependent cellular cytotoxicity (ADCC) in the same controlled autologous system and found that only four out of 20 tested anaplastic glioma patients had positive ADCC responses. Martin-Achard, et al., employed the ADCC assay in a well designed study and concluded that the high frequency of control serum activity (five of 25 or 20%) and the ability to remove...
specific activity from 10 of 60 or 17% of responding patients by platelet absorption was inconsistent with the concept of a specific humoral response of glioma patients to a tumor-associated antigen. Martuza, et al.,10 found no significant responses utilizing the ADCC assay in a patient population of 30.

Therefore, the issue of specific in vitro cytotoxicity via cell-mediated pathways in patients with malignant glial tumors is unresolved.

General Immune Response

In patients with disseminated cancer, cell-mediated immunity has been found to be depressed. Cell-mediated immune responses have classically been assayed by delayed hypersensitivity responses to skin test antigens and by quantitative studies of circulating lymphocytes and/or in vitro functional evaluation of these lymphocytes. Brooks, et al.,14 defined a significant degree of anergy during the preoperative period in patients harboring anaplastic gliomas.

Mahaley, et al.,67 evaluated delayed hypersensitivity responses and lymphocyte counts at the time of surgery and serially in 42 patients with glioblastoma multiforme and 17 others with anaplastic gliomas. At the time of surgery, delayed hypersensitivity responses and the percentage of patients responding to two or more skin-test antigens were subnormal, and the magnitude of cellular anergy was proportional to the presence and extent of anaplasia. Preoperative lymphocyte counts were most reduced in patients with glioblastoma multiforme and slightly reduced in patients with less anaplastic lesions as well. Specific studies regarding the presence of circulating T-lymphocytes have demonstrated a general depression of T-lymphocytes in malignant glioma cases.18 Capability of the cells to respond by blastogenesis in the presence of mitogens has been impaired.27 Young, et al.,146 have studied blastogenesis to the mitogens concanavallin A (Con A) and phytohemagglutinin (PHA) using lymphocytes derived from patients harboring various histological grades of intrinsic glial tumors. Fifty percent of patients with glioblastoma multiforme demonstrated a depression of cell-mediated immunity during this in vitro testing, but lymphocytes from patients with benign astrocytomas did not. Plasma obtained from patients harboring glioblastomas inhibited the blastogenesis of normal lymphocytes to Con A and PHA, while plasma from patients with astrocytomas was not inhibitory. This implies the presence of an inhibitory factor within the sera, a finding consistent with the work of others14,50,60 and demonstrating the credibility of the presence of a blocking factor which was responsible for the impairment of cell-mediated immune responses in glioma-bearing cases. Reduction of tumor burden has been shown to reduce the inhibitory factor titer.14

Studies of the levels of humoral immunity65 have disclosed quantitative immunoglobulin levels well within normal ranges for all patients, with the observation of a higher mean preoperative level of IgM in patients with glioblastoma; however, this relative IgM elevation has not been uniformly disclosed.126

Therefore, in patients harboring malignant glial tumors it is apparent that there is a generalized depression of cell-mediated immune responses and that this depression is related to a serum-blocking factor that has not been specifically identified.

Immunotherapy of Human Gliomas

The major efforts to apply immunotherapy as an adjunct in glioma treatment may be categorized according to accepted immunotherapeutic treatment modes.

Active Specific Immunotherapy

Active specific immunotherapy employs specific immunization of the patient with treated tumor cells, “tumor antigen” preparations, or cross-reacting antigens (viral or bacterial) in an attempt to specifically or selectively augment functional immune response. Thus, the induction of experimental allergic encephalitis (EAE), resulting from an immune response to cross-reactive antigenic components shared by brain and autologous tumor tissue, is a risk to be considered. This is enhanced by the concurrent utilization of adjuvants.

In 1960, W. H. Bloom, et al.,9 attempted to induce an immune response by the subcutaneous implantation of malignant astrocytoma cells in one patient. A total of 15 ml of glioma tissue was injected directly into the subcutaneous tissues of both thighs in 12 injection sites. The tumor grew at 10 of the 12 implantation sites with no evidence of immune rejection. Assays designed to detect alterations of humoral immunity were likewise negative. Autopsy examination disclosed neoplastic cells in regional lymph nodes, with no suggestion of immune rejection. Grace, et al.,36 implanted subcutaneous autografts in six patients with glioblastoma multiforme. Two of the six demonstrated successful growth of the autografts with proliferation of typical glioblastoma multiforme in subcutaneous tissues. Serological studies revealed no evidence of a humoral immune response against brain antigens in any patient. Two patients strongly rejected their tumor autografts and gave positive delayed hypersensitivity responses when tested intradermally with saline-soluble fractions of both normal brain and tumor; however, two patients who
had successful takes of their autografts did not show the positive reactions to skin testing with the same antigens. These results suggest that the presence of delayed hypersensitivity to glioma antigens may have played a role in determining the success or failure of the autografts.

In 1973, H. J. Bloom, et al., reported the results of a randomized prospective clinical trial, using radiated autologous tumor cells in patients with malignant gliomas treated by radical surgery and postoperative radiation. The results in 62 patients showed no statistically significant difference in survival between the group receiving adjuvant plus autologous tumor cells and those treated with surgery and radiation therapy alone. All 27 patients who received irradiated tumor cells were dead by 30 months, whereas five (14%) of the control group survived for more than 3 years, and one patient for more than 72 months. The initial mortality was equally rapid in both groups. Only 10 patients had multiple injections of irradiated autologous tumor cells and none showed positive intradermal skin tests to indicate the development of cell-mediated local reaction against the injected tumor. In the majority of cases in this study, there was clinical evidence of tumor recurrence, which was proved histologically in nine of 10 patients subjected to autopsy. No statement regarding enhancement of tumor growth by immunotherapy could be made on the basis of this study. In addition to the fact that only 10 patients received multiple inoculations of tumor cells, it has become apparent that since the cells administered had been irradiated with 15,000 rads, their antigenicity was significantly reduced. However, recent review of the patients reported by Bloom, et al., has disclosed one possible case of EAE.

Active Nonspecific Immunotherapy

Nonspecific immunotherapy has been used in a small number of cases by Selker, et al., who treated six patients with intrinsic glial tumors with the microbial agent Corynebacterium parvum. No conclusions can be drawn regarding the effectiveness of this therapy in relation to survival time. However, increased intracranial pressure was noted in all the patients with mass lesions and is an important consideration in further efforts to study this agent.

Miki, et al., employed intradermal inoculations of BCG in an effort to stimulate immune responses in 45 patients with primary brain tumors whose reactions to PPD were negative. Survival rate at 3 years after surgery was greater than 50% in 65% (17 of 26) of glioma patients whose PPD skin test was positive following BCG therapy. This figure is impressive when compared with non-BCG-immunized historical controls whose 3-year survival rate was approximately 12%. Patients inoculated with BCG whose PPD reaction remained negative had a rate of survival at 3 years that was comparable to that of the uninoculated controls.

Combination Therapy

Perhaps the best currently documented effort to use combinations of immunological adjuncts is that of Trouillas and LaPras, who immunized glioma patients postoperatively with autologous tumor cells emulsified with complete Freund's adjuvant delivered in four to 10 injections. Sixty-five patients with glioblastoma multiforme were randomly divided into four groups. Seventeen patients had no postoperative treatment, 20 received postoperative radiation therapy, 18 received postoperative radiation therapy and immunotherapy, and 10 patients received postoperative immunotherapy alone. The patients were followed for a minimum of 24 months. Of the 28 patients receiving the immunotherapy, 25 developed cutaneous hypersensitivity responses to autologous tumor cells. Immunotherapy alone significantly increased the duration of remission and survival. Median survival time rose 35%, from 5.4 to 7.4 months. Radiation therapy alone had a similar effect, with a survival time increased to 7.5 months. Combined therapy increased survival time to 10.1 months.

Pathological study undertaken in the postoperative immunotherapy group demonstrated lymphocytic or plasmacytic infiltration of the tumor in four of six cases. The negative samples were taken more than 7 months after immunotherapy, suggesting that the effect was not long-term. Of those patients showing delayed hypersensitivity responses to glioblastoma extract after immunization, dermal reactions were sought with astrocytoma extracts which likewise demonstrated response, but of a weaker nature. During the course of immunotherapy, antibodies were demonstrated that were cytotoxic for glioma cells in culture in a complement-mediated system.

Although apparently impressive, Trouillas' studies lack external pathological review and the small numbers of cases do not permit the stratification of variables which are necessary to draw statistically valid conclusions regarding treatment efficacy. Brooks, et al., have drawn attention to a single case of allergic encephalitis in Trouillas' patients, indicating one of the inherent risks of this form of therapy.

Ommaya and Albright, et al., reported the results of a pilot study designed to evaluate three modes of therapy for patients who had undergone maximum tumor resection and radiation therapy. All patients had a catheter implanted in the tumor for drug ad-
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Administration. The cases were randomized to one of three groups; chemotherapy alone, immunotherapy alone, or immunotherapy plus chemotherapy. Two chemotherapeutic agents were used, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 8-azaguanine. Only two patients received immunotherapy alone, which consisted of the administration of intradermal BCG, intradermal autologous tumor cells (treated with neuraminidase and mitomycin C), and PPD injected into the tumor cyst implant. Patients in the immunotherapy group received both immunotherapy and chemotherapy as described above. This group consisted of only three patients. All five of the patients who had received immunotherapy alone or in combination with chemotherapy developed recurrence of tumor within 12 months. Chromium-51 release cytotoxic assays were performed on three patients with recurrences. The patients' lymphocytes were no more cytotoxic than were normal control lymphocytes. Albright, et al., in evaluation of tumor-related delayed hypersensitivity response, found that some enhancement of cellular infiltrate was apparent in those patients undergoing the immunotherapy. However, in no case was the response more than moderate and in no case did the inflammatory response encompass the tumor at its peripheral margins.

Prospective, well designed, controlled studies are currently underway to evaluate the future of combined therapy in the management of the glioma patient.  

Adaptive Immunotherapy

Takakura, et al., employed autologous leukocytes for direct inoculation into recurrent glioblastoma tumor beds via indwelling catheters or direct intratumoral injection through existing craniotomy openings. All patients treated had failed conventional therapies. Seven of the 17 patients treated had a life expectancy of under 1 month. Autologous lymphocytes were isolated from the peripheral blood and placed in direct contact with tumor cells in an attempt to produce in vitro sensitization. After autologous leukocyte infusions, eight patients sustained gross clinical improvement and were alive up to 17 months later. No neurotoxicity ascribable to the procedure was noted, and particularly no instance of EAE was noted on serial biopsy specimens. One patient who was comatose at the time of a single leukocyte infusion returned to full activity and lived up to 17 months without any increase in tumor mass as evaluated by brain scan. This technique is currently being applied with sensitized macrophages as effector cells.

More recently, investigations have been initiated which employ autologous lymphocyte infusions via an intrathecal route. Pilot studies using metrizamide monitoring of cerebrospinal fluid (CSF) circulation in postoperative tumor-bearing patients have been reviewed in the early stages of developing this technique to define CSF circulation and communications within the tumor bed as well as the rest of the subarachnoid space.

Restorative Immunotherapy

Investigations are currently in progress regarding the utilization of the immunorestorative agents levamisole and thymosin, but data are not available regarding the ultimate effects on tumor course. However, Ommaya has demonstrated that utilization of thymic fractions may have a distinct effect on parameters of in vitro and in vivo lymphocyte function in glioma-bearing cases.

To date, the use of immunomodulation in malignant glioma-bearing patients has met with minimal success. The majority of studies have been undertaken in patients who have failed previous therapies and have extensive tumor burdens. Numbers are too small...
to allow for proper stratification for variables, and studies lack concurrent controls. In addition, monitoring of the alteration of immune parameters has been infrequently undertaken in parallel with the therapeutic effort. Most studies have not undergone independent pathological review to insure uniformity of pathological assessment in conformity to universal standards of evaluation. Current opinion holds that modulation of the immune system will be most effective as adjunctive therapy after cytoreductive therapy by surgery, radiation, and chemotherapy. Under these circumstances, tumor burden can be reduced to a minimum, and thus increase the efficiency of the immune system against the tumor and the likelihood of a cure.

Future Perspectives

If immunotherapy is to be utilized to its maximum potential, clarification of fundamental issues related to immune mechanisms in glioma-bearing patients is of paramount importance. Whenever immunotherapy has been helpful in the treatment or prevention of infectious diseases, it was specific immunization that led to the greatest success. There is every reason, therefore, to explore specific immunization as an approach to cancer immunotherapy. To pursue this approach requires a much fuller knowledge of the tumor-specific antigens. Despite an enormous literature related to the question of tumor-specific antigens, there is no incontrovertible evidence yet advanced for the existence of glioma-specific antigens. Nevertheless, by analogy with other tumors in rodents and man, it is likely that such antigens do exist. The critical issue relates to specificity of reagents. Demonstrating specificity of a serological or cell-mediated reaction is far easier in inbred mice than in heterologous men.

In an effort to lend further definition and perspective in these issues, it is anticipated that hybridoma methodology will be of great value. It is likely that hybridoma-produced antibodies will become powerful tools for the serological analysis of glioma cell-surface antigens, differentiating them from the many other tissue-associated, fetal or phase-specific antigens that have complicated analysis heretofore. These antibodies may play a role in therapeutic approaches as well.

The possibility must be kept in mind that certain antigens may not elicit the production of antibody, and their detection may depend upon methods that measure cellular immunity. Reactivity of lymphoid cells against tumor antigen is probably of greater significance to the host than humoral immunity. In this regard, perhaps the single most important recent development has been the discovery of T-cell growth factor that permits the control growth of sensitized T-lymphocytes in the presence of antigen in vitro. Continuously available cytotoxic T-cells of defined specificity comparable to the serologist’s serum bank in conjunction with improved methods of specificity analysis will lead to new standards of reproducibility and precision in the assessment of immune reactions to the human neoplastic antigens. It may also offer a therapeutic tool for intrasional adoptive immunotherapy or a substrate for production of lymphocyte-derived informational molecules.

Another area of critical importance is the manipulation of humoral and cellular suppressor elements that exert depressive effects on the cell-mediated components of the immune response. Identification and efficient removal of offending circulating suppressor factors in man may lead to the introduction of procedures more specific than plasmapheresis to mitigate their inhibitory consequences on immunity. Techniques involving antibodies to suppressor cells or chemical antagonists to suppressor T-cells and macrophages are currently being perfected. Among these are low-dose chemotherapy selective for precursors of suppressor T-cells, or inhibitors of prostaglandins to antagonize suppressor macrophages.

The attempts at immunotherapy that we have discussed should be considered the first stage of effort. A second stage of immunotherapeutic investigation is at hand in which there are carefully controlled studies with strict monitoring of immune parameters. Information from these efforts should tell us if carefully applied immunotherapy has a role to play in the treatment of cancer. The role of immunotherapy in combined modality treatment regimens with radiation, surgery, or chemotherapy should be defined by these studies as well. A new generation of agents with specific immunological impact will bear close scrutiny; these include: 1) the interferons; 2) glucan, a potent macrophage stimulator; 3) pyran, an interferon inducer and macrophage activator; 4) retinoids, a class of vitamin A derivatives with adjuvant activity and direct effects on differentiation of tumor cells; and 5) thymic hormones.

The term “biological response modification” has been applied to the new approach to immunotherapy. A division has been established at the National Cancer Institute to implement a program in this area. Agents that modify the properties of tumor cells, such as interferon, the retinoids, and intrinsic maturation factors regulating normal cellular development, will be explored as to their ability to alter the host-tumor relationship besides the conventionally used immunotherapy. This broader approach to “biomodula-
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... holds promise for the future in the treatment of this therapeutically difficult group of intrinsic glial tumors.

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