REVIEW OF LITERATURE
Despite many aggressive multimodality conventional therapeutic protocols over the past two decades, the prognosis of patients with primary and metastatic malignant brain tumors remains poor. Furthermore, data from cancer registries from around the world show a persistent increase in the incidence of primary brain tumors particularly in the elderly. This is of concern because recent evidence suggests that the persistent rising trend may not be artifactual. In many cases, systemic malignancies also metastasize to the nervous system with a prevalence that is about twice as common as primary neoplasms in adults.

Immune responses in cancer patients are often far from ideal. Because cancer cells are altered-self cells, one would expect cancerous cells to elicit a cell-mediated response. That is, the immune system should target cancerous cells and destroy them. There are three processes that must occur for tumor elimination. The immune system must "see" the cancer, activate lymphocytes, and the cancer cells must be susceptible to killing. In order for this to take place, lymphocytes should be able to infiltrate to the tumor site. CD4+ T-helper1 (Th1) lymphocytes should then recognize tumor-specific antigens in association with MHC class II molecules on the surface of professional antigen presenting cells and receive signals from costimulatory molecules such as B7. As a result, Th1 lymphocytes should be activated and release appropriate cytokines including interleukin-2 (IL-2), interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α). These cytokines, in addition to stimulation by tumor-specific antigens presented on cancer cell surface MHC class I molecules, should activate cytotoxic "killer" T lymphocytes (CTLs) to lyse cancerous cells. B-lymphocytes should also be activated to secrete neutralizing antibodies that aid in cancer cell phagocytosis by phagocytic cells, although their role in tumor immunity is less important. If any of the processes necessary for the induction of a cell-mediated response fail, tumor elimination may not be effective.

The ideal immune response described above often does not occur in cancer patients because cancer cells evolve mechanisms to evade the body's defenses. The immune surveillance theory hypothesizes that cancerous cells arise
regularly but the body eliminates them before they become harmful to surrounding tissue; only those that evade surveillance develop into tumors. This theory is supported by the increased incidence of cancer in immunosuppressed people such as AIDS patients. If the immune surveillance theory is correct, there is a strong selective pressure favoring cancer cells that can avoid notice or somehow prevent them from being killed by the immune system. The first line of defense against both microbes as well as tumor cells is constituted by the so-called nonspecific or innate immune system, which comprises granulocytes, macrophages and natural killer (NK) cells as its effector cells. With respect to immune surveillance against tumors, macrophages and NK-cells play the key role in recognition and elimination of newly arising cancer cells. Whereas the direct antitumor activity of NK-cells is restricted to certain types of tumor cells, activated macrophages are able to specifically recognize and selectively destroy a wide spectrum of different tumor cell types in a still quite incompletely understood reaction termed Macrophage-mediated Tumor Cytotoxicity (MTC). In addition, these cells, together with the so-called dendritic cells, via the process of antigen presentation, serve as a crucial link between innate and acquired immunity, the second part of the immune system, which is constituted by antigen-specific T and B-lymphocytes. In fact, it has become clear only recently, that macrophages and dendritic cells actually control the induction of specific, T-lymphocyte-mediated immune responses and thus also govern specific immunity against tumor antigens, which is essential for final, long-term eradication of tumor cells in the body.

**Role of T-Lymphocytes**

The main agents of anti-tumoral protection in an organism are the cytotoxic T-lymphocytes or T-killers. On the surface of tumor cells the antigens are represented not only in the form of a single molecule, but also in the form of fragments in complex with Human Leukocyte Antigen (HLA) molecules (Major Histocompatibility Complex [MHC]). The identification of a tumor cells by T-killers depends on the various antigens present in the tumor cells. For T-
lymphocytes the sequence of amino acids of a polypeptide is important rather than how it is packed in the space. In order to get good response from T-lymphocytes to antigens, the antigens should be represented by an antigen-presenting cell (APC).

Common APCs in the body include macrophages, B-lymphocytes, bone marrow derived dendritic cells, Langerhans cells of the skin and human endothelial cells. In the brain, candidate APCs includes preliminary microglia, endothelial cells, capillary pericytes, and occasionally astrocytes and oligodendroglia themselves. Microglia are the most attractive candidates for APCs within the brain, accounting for 5% to 20% of the total cellular composition of the brain27. Microglia are distributed throughout the central nervous system (CNS). APCs express not only the MHC-class II molecule, but also co-stimulatory molecules (eg.- CD28/B7 and CD40/CD40L), both of which are recognized by specific receptors on the T cell. Inside the cell these proteins are split into peptide fragments of length upto 10-20 amino acids. These peptide fragments shift toward the plasma membrane of APC and together with molecules of HLA form receptors, recognizable to T-lymphocytes.

For this purpose T-lymphocytes present their own T-cell receptor (TCR). The TCR consists of peptide complex- α, β, ε, γ, δ, ζ, η each of which are encoded by separate genes. The TCR αβ is a structurally variable disulfide linked heterodimer associated with variant components of the CD3 molecule (γ,δ,ε,ζ)28. The interaction of TCR with CD3 trimers is essential for the intracellular signaling which activates the T cells29. The conjunction of TCR with the tumor peptide represented by the HLA molecule is usually not enough to fulfill the complete action of T-killer. Binding of one more molecule of T-killer i.e. CD8-co-receptor with a HLA molecule of target cell is necessary. Presentation of an antigen to the TCR in the absence of a co-stimulatory signal can lead to T cell anergy, a mechanism potentially responsible for the induction of tolerance of self-antigens and possibly some tumor antigens 30. Identical genes encode TCR of T-killers and T-helpers, but co-receptor for T-helpers is represented by different protein- CD4. The co-receptor-CD4 binds with HLA class II molecule where as the co-receptor-CD8 with HLA
class I molecules. If a macrophage with HLA of the class I represents antigen to an immature T-cell, forms clones of T-killer cells. On the other hand, if macrophage with HLA class II molecules represents the antigen, then form clones of T-helpers. In the human population there are many types of HLA proteins but in each individual present only two types of genes— for HLA molecules of I and II types (MHC class I and II).

The ability of T-killers to respond to tumor cells also depends on molecules MHC of the tumor cells. The molecules MHC of the class I are encoded by the two genes in tumor cells of mouse's: H-2K and H-2D where as in the human tumor cells they are encoded by the following genes: HLA-A, HLA-B, HLA-C. The human HLA-A gene coincides with the H-2K gene of mouse. The CD8 co-receptor of T-killer effectively binds with a molecule H-2K but does not bind with the molecule H-2D.

The more the ratio of molecules H-2K: H-2D on plasma membrane, more the tumor cells become distinct for the T-killers. On the other hand, more the expression of a gene H-2D takes place the more often tumor cells escape from the immune attack by the T-killers in blood stream and accordingly more often metastases are formed.

The rate of expression of genes H-2K and H-2D is variable. The α- and β interferons when injected together induce the expression of H-2D gene, while the gamma interferon increases the expression of H-2K gene. Thus, expression of H-2K and H-2D genes can be regulated and, accordingly, increase the immune response of T-killers against tumors. Besides, the IFN-γ suppresses angiogenesis. T-killer, coming in contact with a tumor cell through their receptors, forms a close bond using Mg⁴⁺ ions, and excretes out protein perforins. Perforins lay out on plasmatic membrane of tumor cell which in the presence of Ca²⁺ gets polymerized forming channels through which enters exceeded amount of water into the cell and finally the tumor cell bursts out. Each T-killer destroys only a limit number of tumor cells after which the deposition of energy and perforins in T-killer cells get exhausted and they die out on their own.
Role of Natural Killer Cells

An important element in the anticancer system is so called the natural killer cells (NK-cells). These cells take active parts in the antiviral and anticancer system of our body. The unique feature of NK cells is that they can destroy the cells in which there are low HLA class I molecule expressions. These cells are not target cell specific, but they have been shown to exhibit several levels of target cell selectivity in the absence of antibody 33.

The natural killer cells have different molecules, on which depend their cytotoxic activities. One of such molecules is CD16 (low-affinity receptor for immunoglobulin G- IgG). The NK-cells can get attached to the cancer cells through antibodies- IgG (if they are present on the cancer cell surfaces) in the presence of CD16 molecules. The other important groups of molecules present on the NK-cell surfaces are CD158 molecules. They are also called the immunoglobulin-like receptors of killer cells (KIR) or the inhibiting receptors of the killer cells (KIR). 12 genes encode the whole sets of these KIR molecules. The different KIR molecules interact with different HLA class I molecules. In other words, the KIR molecules of NK-cells play a role of receptor for the HLA class I molecules of our normal body cells 31.

The inhibiting function in the NK-cells is also fulfilled by CD94/NKG2 molecules, which are rather specific for HLA-E molecules. In difference to KIR, the CD94/NKG2 molecules are related to lectin-like molecules. In general, all the nucleated cells of our organism contain the HLA class I molecules. The expression of these HLA molecules in the cells of our organisms can be changed during tumoral transformations or viral invasions in them. When there are low expressions of HLA molecules, the inhibiting function of KIR molecules in NK-cells remain deactivated and the abnormal cells get destroyed by the natural killers. In this way, the NK-cells protect from the cancer cells with low HLA class I molecule expressions 34.
After the close contact of NK-cells with cancer cells, the NK-cell secrete out protein molecules so called perforins, which lay out on the cancer cell surfaces to form pores. Then the NK-cells stand out from the cancer cells while through thus formed pores start to enter the inter-cellular fluid into the cancer cell. The cancer cell slowly swells out and at the end bursts out. On the NK-cell surfaces there are many stimulating molecules or activators. They are the receptors for IFN-γ, IL-2, IL-12, IL-15, IL-18 molecules. On the surface of all the NK-cells present so called the Fas-ligand molecules (CD178), which can trigger a cell death program in the targeted cells. The activation of NK-cells by IL-2, IL-12 elicits an intensive expression of CD178 in NK-cells. In fact the interaction between CD16 and IgG also lead to the same results. In this way, the destruction of the cancer cells can also be elicited through the interaction of CD178 with the receptors of apoptosis on NK-cells. This is one of the vital reasons why in people with low numbers and/or with low functional activeness of NK-cells develop oncological diseases considerably more frequently.

**Role of Macrophages (MØ)**

Macrophage (MØ) infiltration of tumors is a common observation with many spontaneous and experimental tumors. MØ in contrast to CTL and NK cells may be phagocytic in nature and express CD-68. They also express immunoglobulin Fc receptors and like NK cells, can mediate antibody dependent cellular cytotoxicity; also like NK cells, they can be preferentially cytotoxic for tumor cells upon direct contact in the absence of antibody by causing either cell lysis or inhibition of division. Destruction of tumor cells or inhibition of their growth by activated MØ may be mediated by one or more mechanisms stimulated by MØ binding to tumor cells.

Tumor cells synthesize a factor, which inhibits the migration of macrophages (MIF), which is a very necessary substance for tumoral body growth. MIF fulfils simultaneously two important functions in tumoral body growth. Under the action
of MIF, the macrophages happened to be near the tumor, lose their mobility but their capacity to synthesize bioactive substances is retained. In this way, MIF deprives the macrophages to pass the information to other immune-competent cells about the detection of tumor and allows the tumor to use macrophage as a factory, which produces a huge amount of plasminogen-activators. With the help of the plasminogen activator, synthesized by macrophages, the tumor-cells penetrate into the blood vessels and spread out in the organism.

The macrophages when activated by T-lymphocytes transfer arginine into nitric oxide. This toxic substance kills tumor cells. It occurs as follows: the activated lymphocyte secretes an IFN-γ. It submits a signal whose target is the nucleus of macrophage. This signal stimulates the production of nitric oxide syntheses transferring arginine into nitric oxide. Nitric oxide in turn destroys tumor-cells, reducing the energy formation in Krebs cycle and during the transportation of electrons in mitochondrions. The nitric oxide reduces the synthesis of DNA. In this way, without arginine and the synthesis of nitric oxide macrophage can not fulfill its protective function. Methyl derivatives of an arginine, on one hand, blocks the formation of nitrates in macrophages on the other hand, restrains the ability of macrophages to destroy tumor cells.

**Role of Polymorphonuclear neutrophils (PMN)**

PMNs can also kill tumor cells, reduce their growth rate by either direct contact or by an antibody-dependent mechanism similar to that of MØ and NK cells. Cytotoxicity is generally mediated by activated PMNs. Reactive oxygen products and lysosomal enzymes have been implicated in the killing process. For instance, tumor necrosis factor-alpha (TNF-α) has been shown to induce an oxidative burst among PMNs and H₂O₂ is produced, which has been associated with tumor cytostatic activity. In the immediate vicinity of PMNs, cytotoxicity is non-specific.
Role of B-Lymphocytes

During the interaction of tumoral antigen and B-lymphocyte, a selection of clone B-lymphocytes takes place, whose receptors correspond to the given antigen. They coordinate with tumoral antigens and get activated. In activated B-lymphocytes, processed antigens are located on plasmatic membrane together with MHC class II molecules. Mature T-helper cell, which has undergone specific activation by APC with MHC class II molecule, contacts with activated B-lymphocyte. It helps T-helper to gain the ability to excrete interleukin-2 under whose influences the B-cell undergoes mitosis and differentiation converting into a plasma cell. A mature plasma cell secretes antigen-specific immunoglobulins (antibody). This tumor-specific antibody contacts with specific antigens of a tumor. But the tumor cell has an interesting feature— it can lose the surface antigens (like a lizard loosing its tail when it is caught by it). The total complement level of human being is insufficient to develop an antibody dependant lysis of the tumor cell. The complex antigen-antibody leaves the tumor cell earlier than the activation and polymerization of complement take place. Generated anti-tumoral antibodies and the circulating immune complexes worsen the state of diseases. They block out antigens of tumor cells from receptors of T-killers, protecting tumor cells from cytolylitic attack. However, in most instances, B lymphocytes instead marshal other components of the immune system to defend against invaders.45

On the contrary, the brain has got its own immune system and is composed of neurons and glial cells. The brain remains partially shielded from the peripheral immune system by the blood-brain barrier, which allows selective entry of the activated immune cells to pass through. The activated immune cells also communicate with the resident cells of the intracranium and together initiate an immune response against any pathological condition.
BRAIN "AN IMMUNOLOGICALLY PRIVILEGED SITE"

Old Concept:-

For many years, the brain has been thought to be an 'immunologically privileged site,' where no immunosurveillance of lymphocytes occur. This assumption gained support from the existence of Blood-Brain Barrier (BBB), which excludes components of the peripheral immune system. Additional support of the brain’s immunoprivileged nature is its lack of lymphatic vessels and lymphatic drainage\(^{46,47}\). The BBB is in fact, formed by the vascular interface between the blood stream and the CNS parenchyma\(^{48,49}\). The interface is comprised of endothelial cells surrounded by a basal lamina, pericytes and tightly apposed astrocyte end-feet\(^{50}\). Endothelial cells of cerebral blood vessels from a network of tight junctions\(^{49}\), thus differing fundamentally from endothelia of other organs \(^{51}\). Due to this peculiarity of cerebral blood vessels, a physical barrier exists controlling the exchange of macromolecules and cells between the blood and the CNS \(^{52}\).

Three entirely desperate historical observations established the concept of central nervous system (CNS) immune privilege. The first was Paul Ehrlich’s (1885)\(^{53}\) findings that parentally administered aniline dyes stained virtually all body tissues except CNS; from this arose the concept of blood-brain barrier physiologically separating central nervous tissue from the systemic circulation, and it was later argued from systemic immune responses. The second observation, concerned transplantation, where xeno-and allogeneic tissue transplant into the brain. Murphy & Strum (1923)\(^{54}\), demonstrated that mouse sarcoma tissue was found not to be rejected following implantation into rat brain, implying that the CNS was exempt from the immunological processes responsible for graft rejection. Working on the same line, Engelhardt (1996)\(^{55}\), provided evidence that tissue transplanted from one individual into the brain of another individual survives for extended periods of time. The absence in the brain of direct lymphatic drainage and thus of circulating lymphocytes, deprives the CNS of an apparently essential requirement for immune response generation \(^{56}\). Finally, normal nervous tissue (except astrocytes) was reported not to express major histocompatibility complex antigens constitutively \(^{55,57}\), exempting the brain from participation in cell mediated immune responses.
Modern Concept:-

Current studies, however, showed that the concept of 'brain as immunologically privileged organ' is only partially true. The 'immune privilege' of the brain is in fact determined at the level of Blood-Brain Barrier (BBB). Recent research in this regard has clearly demonstrated that BBB is not a passive protective barrier isolating the brain, but it actually plays an active role in pathophysiological processes occurring within the CNS 46,52.

In inflammatory reactions, in response to viral infections, in multiple sclerosis (MS) and in experimental autoimmune encephalomyelitis (EAE), CNS has been found to exhibit immune response, which further implies the existence of a definite connection between the CNS and the systemic immune system. A regulatory role of IFN-γ in EAE, acting on T cell proliferation and directing chemokine production for the onset and progression of this disease has been found 58. Pacheco-Lopez et al., (2002)59, have suggested that different interconnected brain cell groups respond rapidly to an immune challenge in the periphery, constituting an afferent pathway of neuroimmune challenge. Studies have provided evidence for afferent and efferent pathways of communications between the CNS and the peripheral immune system 60. Afferent pathways of lymphatic drainage of the brain are well established in a variety of diseases. Investigators have shown that injecting antigen into CNS tissue elicits a systemic immune response 31. In examining the efferent pathways by which lymphocytes home to the CNS, several studies have characterized the phenotype of infiltrating T-lymphocytes by use of immuno-cytochemistry 60.

There is now substantial evidence that T-lymphocytes can regularly, although not in great numbers cross the BBB and migrate through the CNS in search of pathogens and recruit CNS cells as antigen presenting cells 47,61. Upon contact with other specific antigens, they initiate local immune response and then in the next stage, cell of the glia and the immune system crosstalk to jointly control the immune reactivity within the CNS 47. Wekerle et al., (1986)62 and Hickey et al., (1991)61 have demonstrated that C14 labeled CD4+ myelin basic protein (MBP) specific T-cell lines, when activated either by presentation of specific antigen or by Concanavaline
A, do migrate to the brain through intact BBB. The first phase of entry occurs within 24 hrs after injection and the second phase occurs 96 hrs post injection. These results suggest that the CNS be under constant immune surveillance resulting in elaborate cellular interactions. Geppert et al., (2001) have also supported lymphocyte infiltration at the tumor site in human glioblastoma. These tumor-infiltrating lymphocytes (TIL) are phenotyped as CD4+ and also CD8+ T lymphocytes in glioma. Geppert et al., (2001) have also supported lymphocyte infiltration at the tumor site in human glioblastoma. These tumor-infiltrating lymphocytes (TIL) are phenotyped as CD4+ and also CD8+ T lymphocytes in glioma. Plautz et al., (2000) have also reported that CD4+ T cells were quantitatively more effective than CD8+ T cells in CNS. CD4+ T cells can mediate an anti-tumor response through paracrine effects of additional secreted cytokines rather than merely serving as a source of IL-2 for CD8+ T cells. Moreover, histopathological and immuno-histochemical studies have also revealed occurrence of PMN and MΦ in the vicinity of malignant brain tumor. Iwatsuki et al., (2000) have shown a percentage of elastase positive PMNs in the infiltrating margin of tumors with greater degree of malignancy. PMNs are recruited to malignant gliomas and the elastase released by these cells aids in the infiltration of gliomas. Macrophages were also identified in untreated, infiltrating Glioblastoma Multiforme. During inflammation or an immune reaction, the organized BBB structure undergoes changes, which allows access of inflammatory cells and other compounds into the CNS. Calcium-activated potassium channels also serve as a convergence point in the biochemical regulation of the blood-brain barrier permeability. Leukocytes migrate across the BBB at sites of inflammation through the specialized endothelium formed cuboidal cells, termed High Endothelium Venule (HEV), similar to those seen in other lymphoid organs. In fact, there are also immune connections in the brain through cervical lymph nodes and also nasal sinuses.

Role of cell adhesion molecules (CAM):-

Leukocytes homing to the CNS are orchestrated by cell adhesion molecules (CAM) on endothelial cells of the BBB and their counter receptor on the immune cells. It has become evident that CAMs are actively involved in mediating the recruitment of specific lymphocyte subsets into different tissues. Several cell
adhesion molecules, such as RANTES, Selectins, Macrophage chemoattractant protein-1 (MCP-1), Macrophage inflammatory protein-1 (MIP-1), intracellular adhesion molecule-1 (ICAM-1), ICAM-2, vascular CAM-1 (VCAM-1) and LFA-3 are expressed on cerebral endothelial cells 74. Over expression of LFA-1, 4, CD-44 and CD-2 molecules on activated T-lymphocytes contribute to adhesion and their ultimate migration across the BBB 55,74.

There are several reports of increased endothelial CAM expression during CNS inflammation. Increased expression of ICAM-1 was described in multiple sclerosis (MS) 46 and experimental autoimmune encephalomyelitis (EAE), the animal model of MS 75. Increased expression of VCAM-1 during the course of EAE strongly correlated with infiltration of T cells, B cells and monocytes into the CNS 75. Similarly, increased expression of addressins, mice endothelial cell antigen (MECA)-325, correlated with inflammatory cell influx into the CNS in EAE 76. More strikingly anti-VLA-4 (α4β1 integrin) effectively prevented development of EAE, suggesting that VCAM-1/VLA-4 interaction is of particular importance in immune cell brain homing in inflammatory autoimmune conditions 46.

**Role of Cytokines:**

In the CNS, cytokines contribute, usually by enhancing immune and inflammatory events, to an array of immunopathological conditions77. Studies with radiolabeled albumin have shown that IL-1β and TNF-α induced an increase in BBB permeability78. A single microinjection of TNF-α and IFN-γ into lumbosacral spinal cord of the rat was shown to produce an inflammatory response79; whereas IL-2 had no effects80. The most striking change observed was gliosis and neovascularization in the vicinity of the TNF-α producing tumor cells81. Adhesion of lymphocytes to brain endothelium increased 3-4 times after incubation of endothelial cells with IL-1 and IL-6 82. IL-1 can increase the expression of ICAM-1 on human astrocytes 83 and is also a strong inducer of cytokine production like TNF-α and IL-6. It has also been demonstrated that IL-1β can induce astrocytes to serve as a source of complement components within the CNS. Activation of the complement cascade by an alternative pathway has been implicated in the destruction of the BBB in
inflammatory conditions of the CNS. Like IL-1, TNF-α enhances ICAM-1 expression and is a potent inducer for other cytokines, IL-6, G-CSF, GM-CSF. In this way, TNF-α might significantly contribute to the attraction of inflammatory cells to the CNS and promote inflammatory processes. Cytokine induced lymphocyte adherence to endothelial cells seems to be due to enhanced expression of adhesion molecules.

The BBB cells not only respond to cytokines but can also be induced to produce cytokines by them. These observations underscore the active role of BBB in pathological conditions. Brain microvessels and pericytes produce IL-1α, IL-1β, IL-6 and GM-CSF.

**Lymphatic drainage:**

Although the CNS does lack a fully developed lymphatic vasculature because there is evidence that CNS extra cellular fluid drains into the deep cervical lymph nodes, the possibility exists that antigen presentation could occur in the CNS. The afferent pathways of lymphatic drainage of brain are well established in rodents. Fluid and antigens appear to drain along perivascular spaces populated by immuno-competent perivascular cells. Drainage pathways connect directly via the cribriform plate to nasal lymphatic and cervical lymph nodes. The drainage of antigen from the brain to cervical lymph nodes, in presence of activated lymphocytes in the meninges or CNS has been shown to result in an increased number of lymphocytes targeting the brain.

**Antigen presentation:**

It has also been shown that in response to inflammatory reactions, neoplasms, or brain injury, the expression of MHC class I and II molecules can be induced on several cells that reside in the brain; such cells include astrocytes, endothelial cells, microglial cells, pericytes and choroid plexus epithelia. Several authors have observed the presence of MHC class II molecules mostly on perivascular cells, astrocytes, and microglial cells. Although TNF-α increases MHC class-1 expression on astrocytes, it has no influence on class II expression.
has also been demonstrated that astrocytes and endothelial cells can present MBP to T lymphocytes, resulting in the lysis of antigen-specific T cells. However, although astrocytes could process and present antigen in the context of appropriate MHC molecules, they are unable to induce T cell proliferation\cite{46}. Studies on antigen presentation by astrocytes and brain endothelial cells are in contrast with the microglial study which demonstrated that human microglial cells can act as fully competent antigen presenting cells\cite{93,94}. In further studies, Frei et. al., (1997)\cite{95}, have also shown that an immunological reaction occurs in the brain in response to a number of disease processes that affect the CNS and spinal cord. Moreover, extensive migration of lymphocytes into the CNS and expression of MHC molecules on vascular endothelial cells, astrocytes, microglial cells and pericytes have been observed in patients with viral encephalitis, multiple sclerosis and allergic encephalomyelitis\cite{95}.

During inflammatory processes, the highly specialized function of the BBB is partially or totally lost and inflammatory cells and other blood-borne compounds have greater access to the CNS. Factors known to disrupt the BBB during inflammation include arachidonic acid, bradykinin, histamines and cytokines. The increased permeability of the BBB during inflammation may be dependent on several factors, one of which is the opening of the tight junctions. In EAE, it has been elegantly demonstrated that tight junctions are opened in endothelial venules\cite{72,96}. Lymphocytes accumulate near to opened tight junctions and migrate through parajunctional channels like structures. Additional structures involved in increased permeability of the BBB are the gaps across endothelial cell\cite{96}. Both the opening of tight junctions and the formation of gaps across endothelial cells seem to be induced by inflammatory cells since they are always associated with the presence of these cells. It has also been demonstrated that canalicular profiles and tracer-positive intracellular vesicles can be identified in animals with EAE\cite{97}. The secretion facilitates the migration into the CNS by lymphocytes of the enzyme heparanase\cite{98} which mediates the degradation of the heparan sulfate proteoglycans of the extracellular matrix. Neutrophils may also contribute to endothelial cell destruction by the production and release of oxygen radicals and proteolytic enzymes\cite{99}. 
Currently, the role of BBB in antigen presentation has also been extensively demonstrated, which can respond to immunomodulator or can induce or produce modulators by themselves. As a result the BBB may participate in the presentation of neural antigen to blood derived immune cells. 46, 52.

Major cell types that participate in executing the immune responses are the glial cells; Microglia, Oligodendroglia and Astroglia. Among them, the microglia comprising of about 20% of the glial population, are the resident cells of the CNS and represents the first line of defense in the brain against infection and damage. 100, 101. Mg represent a highly responsive glial population having potential to engage in recognizing and eliminating invading pathogens, regulating adoptive immunity and participating in pro- and anti-inflammatory mechanisms. Th1 cytokines provide signals for Mg to mature, present antigen and amplify locally the pro-inflammatory immune response. Conversely, Th2 inducing capacity of Mg and astrocytes together with their ability to produce anti-inflammatory mediators could play a role in providing counter regulatory signals limiting CNS inflammation. Therefore, modulation of this master regulator of neuroimmune system can offer an answer against different neuropathogenic condition including brain tumor.

**INTERACTION OF THE GLIAL CELLS:**

Glioc cells of the CNS are postulated to function as immune accessory cells, which may regulate immune reactivity occurring within the CNS, activating or alternatively inhibiting T cell responses. Apart from the function of Mg as the chief immunomodulatory cells of the CNS, these cells also function in sustaining the proliferation of other glial cell types, especially astrocytes. In experimental autoimmune encephalomyelitis (EAE), Mg expresses 'Ia' antigens in the brain with EAE. IFN-γ treated and Ia-expressing Mg also stimulates proliferation of cultured astrocytes under certain conditions. Astrocytes at doses of 3x10⁹ cells to 3x10⁸ cells were cultured with irradiated Mg (1x10⁴ cells). When the two types of brain cells were cultured at a ratio of 1:1, Mg augmented astrocytic proliferation. 103, 104. Rat amoeboid Mg is also found to be able to lyse rat oligodendrocytes in vitro. The lysis
is inhibited by TGF-β, antagonists of NO production, as well as antibodies to TNF-α, ICAM-1 and LFA-1.  

**Astroglia:**

Astrocytes make up a substantial proportion of the CNS glial population and participate in a variety of physiological and pathological processes. Cultured astrocytes are able to present antigens to MHC class I and MHC class II restricted lymphocytes and produce many cytokines. But, more studies have provided ample evidence that Microglia, rather than astrocytes, are the chief immune cell of the brain. However, astrocytes still have important regulatory effects on inflammatory and immune responses directed in the CNS. While MHC class II expression on astrocytes in situ remains controversial, astrocytes stimulated by IFN-γ and TNF-α express MHC class I and MHC class II molecules as well as ICAM-1, VCAM-1 and LFA-3. Depending on the type of T cells, astrocytes have been shown to act as stimulators and inhibitors of the proliferation of primed T cells, and to be able to proliferation of naïve T cells.

**Oligodendroglia:**

Oligodendrocytes are also primary glial cell in the CNS. These cells have been reported to undergo necrosis and/or apoptosis in response to TNF-α, lymphotoxin, general inflammation or radiation. Compared to Mg and astroglia, oligodendroglial cells are particularly sensitive to soluble NO. Exposure to NO also leads to oligodendrocyte cell death. In MS lesions, there is indication of degenerating oligodendrocytes with swollen nuclei and cytoplasm, disrupted plasma and internal cell membranes and other features of necrosis.
MICROGLIA:

NOMENCLATURE

Microglia (Mg) belong to a class of cells—the glia (from Greek, meaning 'glue')—that was first recognized in the 1800s. By the 1920s, microscopists had identified 3 kinds of glial cells: astrocytes, oligodendrocytes and microglia. By the 1970s, it was evident that the cells have profound responsibilities. The motile, polymorphic, microenvironment-sensitive Mg cell capable of transitional phenotypic expression defies the use of conventional descriptive terms appropriate for other fully differentiated cell types of neural tissue.

The term Mg was first used by Rio Hortega in 1919 to describe a type of glial cell that could be differentiated from neurons, astrocytes and oligodendroglia by its distinctive morphology and silver impregnation techniques. Currently, there can be no doubt about the existence of microglia.

Many different names given to Mg due to variability in their appearance depending on developmental stage, functional state, and anatomic location. The two major morphologic subtypes of intrinsic Mg are ramified and amoeboid Mg. Although ramified Mg are sometimes referred to as 'resting' Mg, it is probably inaccurate to ascribe a functional state to this form of Mg based on morphology alone. The descriptive term 'ramified' carries no such implication, merely reflecting a morphologic appearance. Similarly, 'amoeboid' as applied to Mg is sometimes used to indicate an 'activated' Mg. Once again cell shape may not be the best indicator of functional state.

The term amoeboid as applied to Mg means amoeba-like in form, rather than function; however, the constantly changing cell shape of amoeba also may hold true for amoeboid Mg, at least as far as it can be discerned from in vitro studies where microcinematography of brain tissue slice explants has demonstrated amoeba-like motility of Mg as they migrate from the tissue slice to take up a position on the surface of the explant and are readily accessible in patch camp studies. Kuwabara (2003) have recently shown that in vitro, Mg cells exhibit variations in morphology. Mg cells isolated on the 5th and 13th day of in vitro culture have distinct
function. Cell isolated on day 5 were immature cells, termed ‘microglioblasts’, and are characterized by presence of large stomata, large peroxidase and alkaline phosphatase +ive granules and have high proliferative activity and suppressed response to LPS, whereas Mg cell isolated on day 13, were mature cells, devoid of granules and respond to LPS by induction of inducible NO synthase, TNF-α and IL-6.

Brain macrophages, which also are referred to as “lipid phagocytes,” “foam cells,” “compound granular corpuscles” or “glitter cells,” are sometimes loosely referred to as Mg, but brain macrophages are usually found in conditions associated with severe injuries to nervous tissue, including infarcts, demyelinating diseases, and trauma, which lead to disruption of the blood-brain barrier and direct influx of monocytes into the tissue. Isotopic and carbon particle labeling studies have demonstrated unequivocally that the overwhelming majority of brain macrophages are derived from blood monocytes 126. Evidence supporting the idea that some ramified cells can become brain macrophages is the observation of a progressive morphologic transformation of ramified cells into amoeboid cells through intermediate cell types as originally described by del Rio-Hortega 121, and as noted, in particular, in certain demyelinating diseases 127.

ORIGIN AND DEVELOPMENTAL STUDIES OF MICROGLIA

The origin of microglia (Mg) has been a longstanding controversial issue in microglial research, with four major schools of thought, which state that they are derived

I. From invasion of mesodermal pial elements,
II. From neuroectodermal matrix cells,
III. From pericytes, and
IV. From circulating monocytes.

Since the pioneer study of Rio Hortega, who suggested that the Mg cells are derived from pial elements of mesenchymal origin in the embryonic brain, in the course of their migration, these infiltrated pial elements transformed into ramified Mg which persisted through adulthood 128.
Monterio et al., (1996) have reported that both pericytes and Mg are dark cells and present similar ultrastructure indicating that Mg may arise from pericytes and astroglia play an active role in releasing pericytes from the vascular wall. Although, there are still intriguing studies based on analysis of cells cultured from embryonic neural plate at developmental stages preceding vascularization that leave open the possibility of a neuroectodermal origin, more current evidence suggests that Mg are derived from bone-marrow derived precursor cells i.e. monocytes that populate the nervous system after it has been vascularized and assume the form of amoeboid Mg that subsequently evolve to become the ramified Mg. In traumatic brain lesions following an intravenous injection of colloidal carbon as a cytoplasmic marker for monocytes, it was found that carbon-labeled monocytes were the main source of brain macrophages, some of which transformed into Mg during the healing process. These monocytes must enter the developing central nervous system (CNS) from the blood stream, the ventricular space or the meninges. Afterward Mg cells are distributed more or less homogeneously through the entire nervous parenchyma. Stereotyped patterns of migration have been recognized during development, in which long distance tangential migration precedes radial migration of individual cells. The factors that control the invasion of the nervous parenchyma, migration within the developing CNS and differentiation of Mg cells are not well known. These apparently depend on environmental factors such as soluble or cell-surface bound molecules and components of the extracellular matrix.

Along with this was the description of precursor cells in the yolk sac in early development. Kaur et al., (2001) have shown that in embryonic mouse brain, occurrence of lectin labeled precursor cells at the yolk sac was noticed that later appeared in the mesenchymal tissue associated with the neuroepithelium where they penetrate the nervous tissue to become the Mg. Mg progenitors can be detected in neural folds from embryonic day 8. A major finding is also that Mg arises by an intense in situ proliferation comparable to that of neural cells.

Developmental studies have shown that leukocytes can be detected in the developing human brain as early as 8 weeks of gestation. Although active
transformation of these cells into Mg has not been detected, more recent studies of human foetus at later stages of development using immunocytochemical markers and lectin staining methods have demonstrated transitional forms between monocytes, amoeboid cells and ramified Mg consistent with a process of progressive transformation. In developmental studies on human fetuses, Dickson et al., (1997)\textsuperscript{141} have noted a burst of microglial immunoreactivity between 15 and 17 weeks gestational age. Before this time, cells consistent with Mg are more difficult to detect. It must be stated, however, that their studies were based on immunocytochemical analysis with markers to cytoplasmic and surface antigens (eg. HLA-DR or CD 68)\textsuperscript{142}, and it is essential to know that the results do not merely reflect maturation of cell surface markers. Given these caveats, developing Mg seems to emanate from discrete foci, often at interfaces between gray and white matter, especially at the angles of the lateral ventricle near the dorsal and ventral borders of the basal ganglia and thalamus. They initially have a simple morphology with scant cytoplasm, but eventually assume a highly branched shape when found at later gestational stages in gray and white matter more distant from these sites \textsuperscript{141}. Sievers et al., (1994)\textsuperscript{132} tested the hypothesis that some morphological and functional properties of Mg are induced in myelomonocytic cells by nervous tissue, specifically astrocytes by comparing the differentiation of Mg, blood monocytes and spleen macrophages on acellular substrates and on monolayers of astrocytes and fibroblasts in in vitro studies. Their findings indicate that the ramified shape of Mg is induced by astrocytes. Since this morphology can also be induced in blood monocytes and macrophages, we take this to be further evidence for the proposition that microglial cells are derived from the myelomonocytic lineage and, moreover, that property of resident macrophages are largely determined by tissue components of their host organ.

Maslinska et al., (1998)\textsuperscript{143}, studied the morphological forms and localization of microglial cells in the developing human cerebellum by means of immunological marker: Ricinus Communis agglutinin (RCA-1) and ferritin antiserum. Both markers detected the developing microglial cells. RCA-1 recognizes carbohydrate residues on the surfaces of Mg and endothelial cells. Anti-ferritin serum
detected precisely all morphological subpopulations of Mg including amoeboid and ramified cells but endothelial cells remain immunonegative. In 14 weeks of gestations only round amoeboid Mg were ferritin-immunopositive in cerebellum. These cells were localized at the periphery of the periventricular, germinal matrix and were surrounding a group of nerve cell of the developing dentate nucleus. The ferritin positive microglial cells on the convolutions of the dentate nucleus gyri manifested as the cells with short fine-branched processes and scanty cytoplasm. In cerebellum of the 20 week-old fetuses the subpopulation of branched (ramified) microglia were more numerous than amoeboid cells. Amoeboid cells were present namely in the intermediate zone of the future white matter and ramified cells penetrated the inner part of the developing internal granular layer of cerebellum cortex. The upper part of cerebellum cortex was colonized by microglia between 24-28 weeks of gestation. In cerebellum of fetuses over 28 weeks of gestation numerous microglial cells infiltrated mainly the Purkinje cell layer, first in the vermis, later in the hemisphere. From 36 weeks of gestations to the birth ferritin-immunopositive Mg cells gradually disappeared from cerebellar cortex.

Richmann et al., (2002) have reported recruitment of myeloid dendritic cells to ischemic brain lesions and suggest that reactive Mg in remote areas transform into dendritic-like cells.

LIGHT MICROSCOPIC MORPHOLOGY OF MICROGLIA

Microglia (Mg) assume many forms in adult human CNS. The most common appearance of Mg is that of a highly branched, small glial cell within the neuropil. The pattern of branching of Mg cell processes, with secondary, tertiary and even quaternary branches is highly characteristic of this cell type, and distinctly different from the cell processes of astrocytes and oligodendrocytes. Branching occurs in all planes, which makes it difficult to capture the full extent of the Mg cellular domain with routine photomicroscopy.

The cells occur in four different subtypes, ramified, amoeboid, perivascular Mg and rod cells. Ramified cells appear uniformly distributed
throughout the neuropil forming an extensive, non-overlapping reticular array. Each cell appears to occupy a discrete three-dimensional territory. These cells are present in nearly equal numbers in the gray and white matter, where they are sometimes less ramified. Ramified Mg appears uniquely adapted in contrast to other tissue macrophage based on their stability or lack of turnover and mitotic capability. Salimi et al., (2002), have shown that Mg cells cultured for two weeks under basal conditions (serum free, serine free and glycine free medium and poly-L lysine-coated surface), ramification of the isolated cell occurs, which is accompanied by strong down regulation of OX-42, OX-18 and OX-6 reactivity (antibodies recognizing CR-3 and MHC- class I & II respectively).

A form of Mg cell that is not usually seen in normal brain is the rod cell or ‘stab cell,’ so called because of the elongate appearance of the nucleus in routine histological stains. These cells appear to be transitional from between ramified and amoeboid Mg. They have been shown in close physical proximity with the apical dendrites of neurons in specific disease states. They were first noticed by Nissl (1899) and described in host of other conditions. Rio-Hortega (1919) called them cellulus en bastoncito in ‘general paralysis of the insane’. A precondition to the detection of rod cells appears to be injury of neurons that preserves cell structure.

A distinct form of Mg is present in the perivascular space. These cells have more similarities to macrophages than to ramified Mg; hence they are sometimes referred to as perivascular macrophages. Perivascular Mg are located in small vessels (25 to 50 μm diameter) consistent with venules, between the outer basement membrane of the endothelial cell or pericyte and the glia limitans. These cells are not highly ramified, and their cytoplasm frequently has abundant cytoplasmic vacuoles containing pigment and hemosiderin. These cells are capable of presenting antigen in vivo to elicit autoimmune disease. Mandev et al., (1995) reported the presence of MHC-class II molecule (OX-6 and OX-17) on a subset of perivascular Mg in adult rat hypothalamo-neurohypophysial system, suggesting their role in antigen presentation to T-cell. Moreover, several adhesion/accessory molecules are also expressed on these parenchymal cells.
Similar to perivascular Mg, amoeboid Mg share properties with macrophages, which argues that they may be a morphologically less differentiated cell type. It may very well be that the functional repertoire of amoeboid Mg and macrophages is greater than ramified Mg.

**MORPHOLOGY OF Mg IN CELL CULTURE**

Mg isolated and cultured from rodent and human brains usually have the appearance of amoeboid Mg. When tissue is disrupted by pathologic process or mechanical dissociation in the isolation procedure, the surviving ramified cells apparently retract their cell process and revert to a less differential amoeboid cell. The amoeboid cell in culture has an oval to irregular cell profile, with many filopodia and spike-like process. Ramified Mg can be detected in tissue culture given the proper conditions. Development of the ramified form appears to be a function of both the functional states of the Mg cell and the highly ordered environment in which it resides. Where as, they are uncommon in dissociated cell cultures, ramified Mg are more common in explant cultures, perhaps reflecting the structural complexity of the tissue in which the cell grows. Mg grown on long term astrocyte monolayers also undergo a limited amount of ramification. Increasing structural complexity of Mg is also noted in Mg cultures that have been grown in the presence of extracellular substrate molecules, such as fibronectin and laminin, suggesting that contact with extracellular matrix molecules plays a role in determining Mg morphology. Stressed cultures or cultures grown in the presence of macrophage – colony stimulating factor (M-CSF) also show morphologic changes, including increasing polarity and a tendency to grow in pallisading columns or rows. The results indicated that soluble factors are also important determinants of structural phenotype of Mg.

Mg cells in culture are distinct from neurons, macroglial cells and peripheral macrophages by the expression of an inward rectifying K+ channel and the lack of outward currents leads to a distinct physiological behavior in that the Mg cells are very sensitive to depolarizing events. Such a depolarization can be elicited by activation of ATP receptors. Since ATP is released from cells during tissue injury it
has the potential to act as an initial trigger for the transition of one microglial functional state to another.

ULTRASTRUCTURAL MORPHOLOGY OF Mg

Ultrastructural features of Mg in situ include an elongated to oval nucleus that may be quite irregular\textsuperscript{164}. The heterochromatin is irregular, dense, and highly clumped. The euchromatin is sparse and usually denser than that of small neurons. The cytoplasm may be sparse and is usually more optically clear than oligodendroglia. The cytoplasm as few conspicuous organelles, but usually includes a few strands of rough endoplasmic reticulum and a Golgi apparatus. Microtubules are either sparse or absent. Perhaps the most specific histologic features are the presence of lysosomes, lipid droplets, dense cytoplasm, and lipofuscin-like material in cell body and expansions of cell process. Intermediate filaments are not numerous, but vimentin-type intermediate filaments are present, and have been used as a marker for Mg and specifically for brain macrophages\textsuperscript{165}. Detectable actin is present, especially in cultured Mg\textsuperscript{166}, but the membrane cytoskeleton is not obvious with transmission electron microscopy. Glycogen granules are absent, which differentiates Mg from astrocytes with scanning electron microscopy, Mg have ruffled cytoplasm, long filopodia and a few microvilli\textsuperscript{24,167}.

Unfortunately, there are no pathognomonic ultrastructural features of Mg or their processes. It is thus difficult to assess the richness of the microglial reticular array and the density of the microglial cell processes in the neuropil with routine ultrastructural methods. The full extent of the microglial reticular array is best appreciated with immunostaining methods on thick tissue sections using monoclonal antibodies that show no cross reactivity with other neural elements. These methods are superior to the methods develop by del Rio-Hortega since they have immunological specificity that silver stains lack.
LABELING METHODS FOR MICROGLIA

Effects of Fixation and Tissue Processing:

The lack of a unique marker for Mg has made their study challenging. All known markers for Mg are shared with other cell types. Many of the best markers for Mg cell surface antigens are unstable with routine histologic methods. Except for a few markers such as the lectin Ricinus Communis agglutinin -1 (RCA-1)\textsuperscript{168, 169}, markers for Mg are either useless or suboptimal in formalin fixed paraffin sections. Immediate and short duration fixation sectioning with either microtome or vibratome, and staining of free-floating sections has produced the best morphologic definition of Mg. The optimal fixatives have been 4% paraformaldehyde, periodate-lysine-paraformaldehyde and Bouin's solution\textsuperscript{170, 171}.

Macrophage markers:

Many of the best markers for Mg are shared with macrophages. These include in completely characterized macrophage - specific antigens such as those recognized by HAM-56 \textsuperscript{172}, L-35 \textsuperscript{173}, Ki-M1P \textsuperscript{174}, 3AS \textsuperscript{175}, My4, My7 \textsuperscript{12}. These antibodies were raised to cells of monocyte macrophage lineage and but most of the antigens remain to be defined. An antibody raised to Alzheimer brain (AMC 30) has been reported to be specific for reactive microglia in diseased brains, but not Mg in normal brains or macrophages in other organs.

Several different antibodies to CD68, including EBM 11 \textsuperscript{176} and KP-1\textsuperscript{177} recognized Mg, even in paraffin embedded tissue sections. CD68 is not, however, specific marker for macrophages been a 110 kD lysosome associated protein \textsuperscript{178}. The staining pattern of Mg with antibodies to CD68 is usually punctate or finely granular. In cultured Mg, localization of the epitope is on intracytoplasmic membrane organelles or the cytosol adjacent to vacuoles and lysosomes \textsuperscript{179}. Both amoeboid and ramified cultured Mg is immunoreactive for CD68.

Other macrophage markers that are shared with microglia include immunoglobulin Fc receptors \textsuperscript{5,180,181}. Complement 3bi receptor \textsuperscript{145, 156}, various leukocyte integrin molecules \textsuperscript{143} and CD45 RB (leukocyte common antigen - LCA)\textsuperscript{182}. 
of the Fc receptors, those for IgG (FcγRI, FcγRII and Fcγ RIII) appear to have most robust expression in Mg\textsuperscript{183,184}.

**Class II Major Histocompatibility Antigen (HLA-DR):**

The class II major histocompatibility (MHC) antigen, HLA-DR, has been shown to be present on microglia in human brains under a variety of conditions\textsuperscript{5,110,150,185}.

Although previous studies reported class II antigens on astrocytes, more recent studies have demonstrated that most of the cells in the brain with class II antigens are Mg, and not astrocytes\textsuperscript{186}. The detection and induction of class II antigens on cultured astrocytes, however, is well established for rodents\textsuperscript{187}.

Detection of HLA-DR on human Mg in autopsy brain tissue appears to be dependent on a number of technical factors\textsuperscript{171}. There are several commercial antibodies to HLA-DR, and they are not equally useful for detecting Mg. LN-3, an antibody recognizes non-polymorphic antigens in the DR region, and gives more reproducible staining of Mg in tissue then antibodies to more polymorphic epitopes. Detection of HLA-DR immunoreactivity also is clearly a function of clinical factors, such as the age and agonal state. In particular, HLA-DR appears to be increased in brains of elderly humans who have died of infections or inflammatory conditions, such as septicemia or disseminated malignancy\textsuperscript{171}. In rodents also, studies suggest that class II MHC immunoreactivity is increased in microglia of old animals\textsuperscript{188}. Other microglia markers, such as the β-2 integrins and LCA, are constitutively expressed in microglia from brains of all ages\textsuperscript{171,189}. These results suggest that HLA-DR may be constitutively expressed at low-levels, but induced or upregulated in a variety of conditions. Since increased microglial HLA-DR immunoreactivity is noted in systemic inflammatory disease (including AIDS), where increased levels of interferon gamma (IFN-γ) are detected and since IFN-γ is known to induce HLA-DR in cultured Mg\textsuperscript{112}, it is reasonable to speculate that HLA-DR may be upregulated in these disorders in response to soluble systemic factors such as IFN-γ.

In experimental models, class II MHC antigens have been shown to be upregulated in the target zone of denervated or injured neurons\textsuperscript{190,191}. They also can
be induced by either local brain injection or intraperitoneal injection of IFN-γ. Taken together, these results suggest that expression of MHC class II antigen may be a marker for microglial activation. In cultured human foetal microglia, HLA-DR is downregulated by M-CSF. Further studies are obviously needed to determine the function of HLA-DR in microglia.

MHC-class II antigens are involved in antigen presentation to lymphocytes and studies in rodents have indicated that perivascular Mg and amoeboid Mg can present antigen, but ramified Mg do not appear to be functional antigen presenting cells. The significance of HLA-DR on ramified Mg is open to speculation.

**Lectin histochemistry:**

Lectins, in particular Ricinus Communis agglutinin 1 (RCA-1), have been used to label human Mg but they also label other cells, most notably endothelial cells. Lectin binding reflects interaction of a specific class of plant protein with specific cell surface glycosubstances, some of which may be important for cell recognition and adherence. Mg from different species has different lectin binding properties. The beta-D-galactose molecule seems to be relatively specific to human Mg. It is not a specific marker for Mg but has been widely used as a marker for microglial reactions in a wide variety of disorders since it was initially described.

**Enzyme histochemistry:**

Mg can be detected with histochemical methods that take advantage of their intrinsic enzymatic activities. In particular, amoeboid Mg like other macrophages, contain nonspecific esterase, which is not, however, convincingly demonstrated in ramified Mg. Conversely, ramified Mg can be detected with enzyme histochemistry for nucleoside diphosphatase. Although this method labels the plasmalemma and Golgi membranes of Mg, it unfortunately also labels other cell types, including some neurons. A variety of other histochemical markers of Mg have been described including acid phosphatase, aryl sulfatase, ATPase, peroxides, 5′-nucleotidase.
Other Markers:

Some of the markers for human Mg are incompletely characterized antigens common to cell types other than macrophages. One of these markers is a monoclonal antibody to a B-lymphocyte antigen, LN-1\textsuperscript{202}. LN-1 recognizes a sialo-gycosubstance, but the actual molecule in Mg has not been defined. This microglial marker is highly sensitive to the state of tissue fixation\textsuperscript{170}. In tissue that is fixed briefly, such as acetone fixed cryostat sections, LN-1 stains both astrocytes and microglia, whereas in more extensively fixed tissue, only Mg is labeled. A number of growth factors act through cellular kinases that specifically phosphorylate tyrosine residues. Antibodies specific to phosphotyrosine residues have been generated. Anti-phosphotyrosine antibodies and antibodies to phosphotyrosine phosphatases have been used to label Mg in rodents and humans\textsuperscript{203, 204}. In experimental conditions associated with microglial activation, phosphotyrosine immunoreactivity is increased\textsuperscript{205}. Based on these observations, it has been suggested that tyrosine phosphorylation is involved in signaling events associated with microglial activation.

Among the other molecules that have been detected in Mg, usually with immunocytochemical methods, none is specific. Heat shock or small stress proteins, including αβ crystalline, have been reported to react with Mg in disease states, but they also stain other glial cells\textsuperscript{206}. Antibodies to certain proteases, in particular to matrix metalloproteinases or collagenases also label Mg\textsuperscript{207}. A novel finding that has not been completely explored is the possibility that there may be specific forms of glucose transporters in Mg. Human Mg has been specifically stained with antibodies to GLU-4\textsuperscript{208}.

ROLE OF MICROGLIA IN THE IMMUNE SYSTEM

During the past decade, evidence has accumulated that Mg plays a major role in host defense. Although, their function in the CNS remains elusive, these cells serve major homeostatic and reparative functions as evidenced by their prompt response to physiological and stress stimuli as well as by their ability to secrete cytokines and neurotrophic factors and become phagocytic when neurons are damaged\textsuperscript{209, 210}. After CNS infection, exposure to inflammatory stimuli, or interaction with blood-derived
cells, Mg become activated to perform several innate immune functions, including induction of inflammation, cytotoxicity, and regulation of T-cell responses through presentation of antigen. Extensive research carried out in *in vitro* systems has provided support for the concept that Mg is involved in CNS immune surveillance.

In recent years, several articles have reviewed molecular and functional aspects of reactive microglial responses in various pathologies, ranging from axonal injury, ischemia, tumors, traumatic damage and neurodegenerative diseases, to infection and autoimmune CNS diseases.

**REGULATION OF MICROGLIA IMMUNE FUNCTIONS**

Despite sharing a common monocytic progenitor with macrophages present in other tissues or associated with the CNS, the ramified Mg found in the normal adult CNS display a downregulated (or perhaps less differentiated) phenotype characterized by lack of endocytic and phagocytic activity, low expression of the leukocyte common antigen (CD45), and low to undetectable levels of membrane ligands and receptors that are essential for mediating or inducing typical macrophage functions. One of the most remarkable properties of Mg is to respond promptly to signals from the inside, as well as from the outside, and to direct their response for purposes of tissue repair and induction of protective immune responses. To carry out these diverse sets of functions, microglia need to go through a process of maturation or activation, leading to the acquisition of macrophage differentiation markers and effector properties, the latter in turn being strictly dependent on the type of inducing stimulus.

**SIGNALS FROM THE INSIDE i.e. CNS ENVIRONMENT**

The neuron-to-microglia communication plays a key role in shaping the quiescent and reactive states of Mg. Numerous receptors for CNS signaling molecules (ATP, neuropeptides, neurotransmitters) and neurotrophic factors have been demonstrated on Mg, which suggests that these cells not only monitor but are also under the strict control of the neurochemical environment. The effects of this...
neurochemical environment on the Mg are site-specific and this could account for differences in the degree of Mg activation and inflammatory reactions in different CNS regions. In vitro studies have provided evidence that substances released during normal neuronal activity counteract the effects of classical macrophage/microglia activators such as the Th1 cytokine interferon-γ (IFN-γ) and the bacterial endotoxin lipopolysaccharide (LPS). Neumann et al., (1996) have first shown that electrically active neurons inhibit IFN-γ induced expression of major histocompatibility complex (MHC) class II molecules on astrocytes and microglia. Later, neurotrophins (NGF, BDNF, NT-3) have been shown to inhibit Mg expression of MHC class II and costimulatory (B7-2/CD86 and CD40) molecules implicated in antigen presentation. Nor-epinephrine and neuropeptides, such as α-MSH, VIP and pituitary adenylyl, such as activating polypeptide, inhibit production of proinflammatory cytokines and nitric oxide in LPS-activated Mg, mainly by raising intracellular cyclic adenosine monophosphate (cAMP) levels. Conversely, some neurotransmitter molecules, like substance P and ATP, seem to enhance the proinflammatory phenotype of microglia, suggesting the existence of a complex interplay between local inhibitory and stimulatory influences in shaping microglial responses. Aloisi et al., (1997) and Vincent et al., (1997) have shown the role of astrocytes in downregulating the microglial secretory activity, possibly through production of TGF-β.

In very recent studies, a previously identified membrane-bound glycoprotein termed OX2 (CD200) was characterized as an important regulator of macrophage populations in different tissues. The investigators have shown that OX2, which is expressed on neurons, lymphoid cells, and endothelium, recognizes another membrane glycoprotein, termed OX2 receptor (OX2R, CD200R), which is restricted to cells of the myeloid lineage, including dendritic cells, macrophages and microglia. In OX2-deficient mice Mg exhibit an activated phenotype (less ramified morphology, enhanced expression of CD45 and complement type-3 receptor, CR3) and upon facial nerve transaction, show an accelerated reactive response. These results clearly implicate neuronal bound OX2 in delivering an inhibitory signal for Mg cells.
providing the first in vivo demonstration of neuronal control of Mg function through cell-to-cell interactions. A better understanding of the OX2-OX2R pathway could provide further insight into the mechanisms implicated in Mg activation in neurodegenerative (e.g., Alzheimer disease) and neuroinflammatory (e.g., Multiple sclerosis) diseases.

Ogura et al., (1994)\textsuperscript{230} have provided evidences that OX-42 stains Mg in both activated and resting states whereas OX-6 stains Mg mainly in the activated state.

**RECEPTORS FOR ACTIVATION SIGNALS FOR Mg**

The prompt response of Mg to a variety of infectious and inflammatory stimuli is their constitutive and inducible expression of a large array of surface receptors that trigger or amplify innate immune responses. These include

a) Pattern recognition receptors implicated in the recognition of pathogen-associated molecules.

b) Complement receptors.

c) Cytokine receptors and
d) Receptors that enhance macrophage effector functions after interaction with the adaptive immune system e.g., T cell or Ig.

A.

*Pattern recognition receptors:*

Several articles have provided evidence that phagocytic, cytotoxic and proinflammatory functions are induced in Mg in a number of CNS infectious diseases (HIV-1 infection)\textsuperscript{211, 216, 231}, or after in vitro exposure to a wide variety of pathogens (viruses, gram+ve & gram-ve bacteria, parasites) and pathogen components (viral proteins, bacterial cell wall components or DNA)\textsuperscript{232-236}. A set of pattern recognition receptors interacts directly with microbial structures leading to stimulation of phagocytosis, induction of cytotoxic mechanisms and activation immunogenes\textsuperscript{237}. Among these, the integrin CD11b/CD18, also known as complement type 3 receptor (CR3), the mannose receptor, and the LPS
receptors are expressed by macrophages within the meninges, choroid plexuses and perivascular spaces, whereas resting Mg express low to moderate level of CR3, but no CD14 or mannose receptor. Becher et al., (1996) has previously reported that CD14 becomes expressed at high levels on the cells. CD14 is an apparent marker of Mg activation, which is not based on changes in morphology or APC capacity.

Zimmer et al., (2003), have recently reported the expression of mannose receptor by Mg.

CR3, through its lectin site, binds an array of microbial molecules and acts as a powerful mediator of phagocytosis and activator of a variety of intracellular signaling pathways, including kinase cascades that are critical in leukocyte activation. CR3 is one of the earliest markers to be upregulated on activated Mg and its increase has been reported in virtually all-pathological conditions. The mannose receptor, which has been implicated in endocytic clearance of host-derived glycoproteins as well as bindings and phagocytosis has been detected only on cultured microglia, but also on resting Mg. IFN-γ treatment led to a decrease and IL-4 to an increase of mannose receptor expression Mg.

LPS, a major constituent in the outer membrane of gram-negative bacteria, binds to CD14 and has been used extensively to activate microglia, both in vitro and in vivo. LPS is the major inducer of Mg production of pro-and anti-inflammatory cytokines, chemokines, prostaglandin and nitric oxide. CD14 is readily upregulated on Mg upon culturing and in vivo after LPS and TNF-α treatments. Kaisho & Akira (2000), recently indicated that LPS-induced signal transduction begins with CD14-mediated activation of Toll-like receptor (TLR)-4. TLR-4 belongs to a family of receptors that share a cytosolic domain, the Toll/IL-1 receptor (TIR) domain, and are involved in host defense and inflammation. The TIR super family includes the type 1 IL-1 receptor (IL-1RI), the IL-18R, and 12TLRs representing recognition and signal
transducing receptors for microbial molecular components. Among these, TLR-2 recognizes products such as peptidoglycans and lipopeptide from gram-positive bacteria and zymosan from yeast, TLR-4 is the receptor for LPS, and TLR-9 recognizes bacterial DNA. Stimulation of cells by IL-1, IL-18 and LPS induces similar intracellular events (e.g., NF-κB, MAP kinase and JNK kinase activation) and requires similar signaling pathways. So far, activation of TIR signaling molecules, like IRAK and TRAF-6 has been demonstrated in IL-18 stimulated cultured Mg. Transcripts for TLR-2 and TLR-4 are expressed in the brain and are differentially regulated by LPS, but studies on the expression of different receptors of the TIR superfamily in reactive or cultured Mg are still lacking.

Nakajima et al., (2003) have reported that LPS-stimulated Mg require priming by protein kinase C (PKC) activation for the induction of harmful factors (like release of NO and TNF-α secretion), while a part (30%) of original PKC activity is sufficient for durable Mg activation.

B. Fc and Complement Receptor

Of critical importance associated with Mg activation is upregulation of opsonic receptors, which mediated or enhance phagocytosis through recognition of serum components deposited on microbes or altered host components. These includes Fc-γ RI, II and III, which bind the constant fragment of immunoglobulins; CR1, CR3 and CR4 binding the complement component C3b; and C1qRp, binding C1q. All these receptors are expressed on resting microglia. In cultured Mg, ligation of CR1, CR3 and Fc receptor triggers phagocytosis of opsomized targets, whereas ligation of C1qRp enhances FcR-and CR1-mediated phagocytosis. Fc and complement receptors may mediate target phagocytosis by Mg in all neuroinflammatory conditions associated with specific humoral responses against CNS pathogens or self-antigens and appropriate activation of complement, respectively. Because signaling
through CR3 and Fc receptors promote production of cytokines and reactive oxidants, these receptors may also enhance Mg proinflammatory and cytotoxic functions.\textsuperscript{154,259,260}

Evidences are emerging that opsonic receptors, pattern recognition receptors and other recognition receptors (e.g., scavenger receptors and phosphatidylserine receptor recognizing lipids on apoptotic cells) expressed on the surface of phagocytic cells, including Mg\textsuperscript{261,262}, may co-operate to ensure efficient elimination of pathogens or infected cells and induction of adaptive immune responses\textsuperscript{263,264}. Elucidation of the complex array of recognition/phagocytic receptors and related signaling mechanisms in Mg may shed light into the functional meaning of microglial responses in different pathological settings.

In infection and autoimmune CNS diseases, Mg also upregulate expression of receptors for the complement anaphylatoxins C3a and C5a, which may be implicated in Mg chemotaxis and activation\textsuperscript{265}.

C. \textit{Cytokine receptors:}

Microglia also express receptors for a number of cytokines that are produced intracerebrally during CNS inflammation. These include receptors for pro-and anti-inflammatory cytokines whose balance should be determinant in inducing and regulating Mg immune functions.

Interferon-\(\gamma\) (IFN-\(\gamma\)), a cytokine produced mainly by natural killer (NK) cells and T-helper (Th1) subset of CD4\(^+\) T cells, is the best known inducer of Mg antimicrobial, proinflammatory, and antigen-presenting functions\textsuperscript{255,266-268}. IFN-\(\gamma\) receptors appear to be constitutively expressed on resting Mg, as intracerebral injection of IFN-\(\gamma\) readily induces Mg MHC-class II expression\textsuperscript{89}. Binding of IFN-\(\gamma\) to its receptor on cultured Mg induces transcription of many immune genes via the classical IFN-\(\gamma\)-induced signal transduction cascade, involving activation of the transcription factor STAT-1 and its binding to IFN-\(\gamma\) activation site (GAS)
elements in the promoter region of IFN-γ responsive genes. Tumor necrosis factor α (TNF-α) is another proinflammatory cytokine with macrophage activating function, which has been shown to promote microglia phagocytosis as well as production of pro- and anti-inflammatory cytokines. During CNS inflammation, TNF-α is produced by activated Th1 cells and also Mg, and probably represents a major autocrine activator. TNF-α binds to two structurally related receptors, TNFR I and TNFR II, whose expression has been detected in Mg in vitro, and in the brain of HIV-infected individuals. TNF-α induces transcription of many immune genes by activating a number of transcription factors, including NF-κB. NF-κB is activated in numerous Mg and CNS-infiltrating macrophages, in multiple sclerosis and experimental autoimmune encephalomyelitis, and may be involved in rapid response of these cell types to inflammatory stimuli, including TNF-α and direct interactions with encephalitogenic T cells.

Several in vitro studies have indicated that Mg respond to IL-1 (IL-1RI and IL-1RII) with increased production of TNF-α and chemokines. These effects are most likely mediated by IL-1RI, as the IL-1RII appears to be a non-signal-transducing “decoy” receptor. Receptors for other immunoregulatory cytokines, like IL-12, IL-16, IL-18 have been detected only on cultured Mg, but effects of their stimulation on microglial functions remains elusive.

Most of the Mg immune functions induced by IFN-γ and LPS are inhibited by anti-inflammatory cytokines like IL-4, IL-6, IL-10, IL-13 and TGF-β. Later workers have further elucidated the molecular mechanisms through which the above cytokines interfere with IFN-γ induced expression of several immune genes in Mg.
D. **CD40-CD40L pathways:**

CD40 molecule, a member of the TNF-R superfamily, is found on the surface of APC, like B lymphocytes, dendritic cells, and activated macrophages, whereas its natural ligand, CD40L or CD154, is expressed on activated T lymphocytes. The CD40-CD40L system has a crucial role in the development of many immune responses, particularly in promoting differentiation of activated B cells, as well as productive interactions between T cells and APC. Ligation of CD40 by CD40L stimulates APC to express costimulatory molecules that are needed for T cells to become fully activated, and to produce pro-inflammatory cytokines, chemokines and cytotoxic radicals. CD40 signaling occurs mainly through activation of NF-κB and this pathway plays a crucial role in the induction of EAE. Gerris et al., (1996) have identified CD40 expressing activated microglia and CD40L expressing T cells in MS and EAE brain lesions. IFN-γ and LPS or Th1 cells interaction induces CD40 expression in Mg. CD40 ligation on Mg stimulates Th1 promoting cytokine IL-12 production. These findings suggest that in inflammatory conditions where antigen-specific T cells invade the CNS and interact with antigen presenting Mg, the CD40-CD40L pathway may mediate activation of both cell types, thereby promoting intracerebral inflammation. Tan et al., (1999), have further shown that CD40-CD40L interaction induces TNF-α secretion and neurotoxic properties in Mg primed with β-amyloid, implying that this pathway may be involved in the pathogenic cascade. Significant levels of TNF-α and IFN-γ were also found in the medium of co-cultured activated CD4+ T cells and Mg cells, pointing to the fact that Mg can supply the CD40 receptor to activated CD4+ T cells.

IFN-γ is the most potent inducer of CD40 expression in Mg, and this induction is mediated by the IFN-γ activated transcription factor STAT-1α and it involves the production of TNF-α.
MICROGLIA AS SOURCE OF INFLAMMATORY AND IMMUNOREGULATORY MEDIATORS

❖ CYTOKINES:

Cytokines are key regulators of innate and adaptive immune responses. Microglia (Mg) along with other immune cells have been detected as sources of cytokines affecting CNS-specific inflammation. However, both in disease conditions and in culture systems, Mg, rather than astrocytes, appear to be the principal source of critical pro-inflammatory (IL-1, TNF-α) and immune regulatory (IL-12, IL-18) cytokines.

IL-1 and TNF-α are two important proinflammatory cytokines with pleiotropic and largely overlapping functions, produced by Mg and blood-derived macrophages during CNS inflammation. In cultured Mg, synthesis and secretion of both cytokines are induced by LPS and in case of TNF-α, are enhanced by IFN-γ. These cytokines have the ability to induce expression of adhesion molecules and chemokine synthesis in cerebrovascular endothelial cells and astrocytes, which facilitate leukocyte extravagation and recruitment into the CNS. Akassoglou et al., (1998) have further suggested that intracerebrally produced TNF-α may be involved in initiating CNS tissue destruction and inflammation.

In vitro evidences has established that Mg are the major CNS sources of pleiotropic cytokines that stimulate cell mediated and humoral immune responses. These include IL-6, a cytokine with both pro- and anti-inflammatory actions, and promote B-cell growth and differentiation. IL-12 and IL-18, IFN-γ inducing factor, have a critical role in stimulation of natural killer (NK) and Th1 cells; and IL-15, which selectively activates NK and CD8+ T cells. A number of workers have shown that except IL-15, all the above cytokines are produced within the CNS during infections and autoimmune diseases and are critically involved in the development of EAE. IL-15 interacts with complement of the IL-2 receptor (IL-2R) and exhibits T-cells stimulating activity similar to that of IL-2. IFN-γ and LPS increased IL-15 production in Mg.
Normal non-stimulated human Mg expressed constitutively mRNA transcripts for IL-1β, IL-6, IL-8, IL-10, IL-12, IL-15. Evidences have also been provided that Mg produces anti-inflammatory cytokines like TGF-β, IL-10 and IL-1 receptor antagonist (IL-1ra). IL-1ra has a major role in counter-acting the biological effects of IL-1, owing to its ability to bind to IL-1RI without initiating signal transduction. Human Mg also expresses mRNA transcripts for IL-1R II, IL-5R, IL-6R, IL-8R, IL-9R, IL-10R, IL-12R, IL-13R and IL-15R. Both TGF-β and IL-10 inhibit macrophage/Mg activation by down-regulating the expression of molecules associated with antigen presenting and production of proinflammatory cytokines, chemokines, nitric oxide and oxygen radicals. All these cytokines suppress Th1 mediated immune responses and have protective effects in the development of EAE.

The balanced interplay between pro-and anti-inflammatory cytokines is crucial in the escalation and resolution of the inflammatory cascade, so it would be interesting to know how their production is regulated in Mg. In vitro studies suggest that IFN-γ inhibits Mg production of IL-10 and IL-1ra, whereas anti-inflammatory mediators like prostaglandin E$_2$ (PGE$_2$) and IL-4 have opposite effects. Mg cells secrete negligible amount of IL-10, but co-culturing them with activated T cells results in significant IL-10 production. This generation was cell-contact dependent and treatment with anti-CD40, B7 or anti-CD23 decreased IL-10 content in Mg-T-cell co-culture. IL-10 decreased the expression of both IL-6 receptor and LPS-induced IL-2 receptor but not IL-4 receptor on Mg. In humans also, Mg produces IL-10 following LPS, IFN-γ stimulation. Functionally, recombinant human IL-10 downregulates basal HLA-DR expression by Mg and inhibited in a dose-dependent manner, the ability of Mg to stimulate CD4+ T cells. IL-4 and IL-10 enhanced the entry and replication of HIV-1 in Mg through upregulation of CD4 and CC chemokine receptor 5 (CCR5) expression. Further, in human cytomegalovirus infection, downregulation of CD4 in microglial cells was observed.
CHEMOKINES:

The critical step in the development of host defense responses to neurotrophic pathogens as well as in the induction and maintenance of CNS autoimmunity is the recruitment of leukocytes from the blood compartment into the CNS. In this process, chemokines produced within the neural tissue play an important role. Chemokines are a superfamily of small peptides (based on a cysteine motif, four major families are identified, CXC, CC, C and CX3C), which interact with seven transmembrane domains, G-protein-coupled receptors expressed on a wide variety of immune and non-immune cells. During CNS inflammation, Mg and other immune cells like astrocytes, endothelial cells are potential sources of intracerebrally produced chemokines. Microglia produce chemokines of the CXC or α family (IL-8, IP-10) and of the CC or β family (MIP-1α, MIP-1β, MCP-1, RANTES), and may contribute to the intracerebral recruitment of T cells, macrophages and dendritic cells. Mg also produces chemokines like MDC and MIP-3β after in vitro exposure to IFN-γ. These chemokines are constitutively expressed in lymphoid organs. MDC is chemotactic for immature dendritic cells and memory/differentiated T cells, where as MIP-3β induces migration of mature dendritic cells and naïve T cells. Thus, these chemokines promote the co-ordinated recruitment and interaction of dendritic cells and T cells and this, in turn is a crucial event in the progression of autoimmune inflammation, allowing continuous local presentation of CNS antigens by APC as well as generation of new auto-reactive T cells within the CNS itself.

Expression of chemokine receptors has also been demonstrated on Mg in vitro and in different pathological disorders. These include: CCR2, receptor for MCP-1, MCP-3, and MCP-4; CCR3, receptors for MCP-3, MCP-4 and RANTES; CCR5, receptor for MIP-1α, MIP-1β and RANTES; CXCR4, receptor for SDF-1, and CX3CR1, receptor for fractalkine/neurotactin, a chemokine normally produced by neurons. Cultured Mg migrates in response to fractalkine, MIP-1α, MIP-1β, MCP-1 and RANTES, indicating a signaling function for most of the above receptors.
PROSTANOIDS:

Another important regulators of inflammation and immune responses are the prostanoids (i.e. prostaglandins and thromboxanes), which are synthesized from arachidonic acid through the cyclo-oxygenase (COX) pathway. Evidence has been provided that both Mg and astrocytes produce large amounts of PGE_2 in vitro. Cultured Mg also produces PGD_2 thromboxane B_2. COX-2 (isoform) immunoreactivity associated with Mg in CNS infections diseases, and with Mg and astrocytes in EAE. PGE_2 is thought to have a protective role in inflammation due to its ability to inhibit macrophage proinflammatory functions and to downregulate Th1 responses. It also inhibits Mg production of pro-inflammatory cytokines and nitric oxide as well as expression of MHC class II and costimulatory molecules. PGE_2 effects on Mg occur through binding to the EP_2 receptor and elevation of intracellular CAMP levels. These findings suggest that prostanoid production by activated cells may be another factor that contributes to the local regulation of inflammatory and immune responses. 15d-PGJ_2, another member of the prostanoid family, has been shown to act as a potent inhibitor of Mg pro-inflammatory functions in vitro by binding to the peroxisome proliferator-activated receptor-γ (PPAR-γ).

ANTIGEN PRESENTATION TO T-CELL; CELL-MEDIATED IMMUNE RESPONSES

Antigen presentation is one of the most critical events in cell-mediated immune responses. It is involved in the generation of protective T-cell responses against infectious agents or tumoral cells, and of pathogenic, autoreactive T-cell responses against self-components. It requires interaction between the T-cell receptor and processed antigenic peptides bound to major histocompatibility complex (MHC) molecules on the surface of APC. MHC class I and class II molecules stimulates CD8+ (Cytotoxic) and CD4+ (helper) T-cells respectively. Additional interactions between adhesion molecules (LFA-1 [CD11a], LFA-3 [CD58], ICAM-1 [CD54]) and costimulatory (CD40, B7-1 [CD80] and B7-2 [CD-
molecules expressed on the surface of APC and specific counter-receptors on T cells are required for optimal T-cell-APC adhesion and reciprocal activation \(^{286,340}\).

In the normal CNS, expression of MHC class I and class II molecules is mainly confined to dendritic cells and macrophages of the meninges, choroid plexuses and perivascular space. APC residing in their CNS compartments have been shown to play an important role in the induction and regulation of T cell responses against microbial and cell-antigens\(^{195,341}\). In normal CNS parenchyma, MHC expression is generally minimal or absent, and when present, it is restricted to some Mg \(^{24,212,342}\). These observations possibly indicate that in their resting condition, Mg behave as poor APC \(^{195}\). Ample evidence has been provided that Mg readily upregulate MHC class II expression in virtually all neurodegenerative and inflammatory conditions\(^{343}\). Further, during CNS inflammation, activated Mg also express other adhesion and costimulatory molecules like CD11a, CD40, CD54, CD58, CD80 and CD86 \(^{344-347}\). Three different integrins, each with a unique \(\alpha\) chain (CD11) and common \(\beta2\) chain have been detected in human Mg: CD11a/CD18 (LFA-1), CD11b/CD18 (MAC-1 or CR3) and CD11c/CD18, (CR4, p150.95 or Leu-M5) \(^{180}\). In particular, interaction of CD80 and CD86 molecules with CD28 expressed on T cells is required for inducing T cell cytokine secretion, growth and survival \(^{346}\). Moreover, CD40 ligation by CD40L on T cells enhances MHC class II and CD80/CD86 expression as well as cytokine secretion by APC, which in turn promotes T-cell activation \(^{286}\). In infections and autoimmune diseases, in which T cell responses are initiated in lymphoid organs, antigen-specific interactions may be established between CNS-recruited T cells and Mg. Such interactions could be relevant for the intracerebral reactivation of T cell recognizing processed viral antigens on infected Mg, eg, during HIV-1, HTLV-1 and measles virus infections \(^{348}\). In multiple sclerosis, both Mg and blood-derived macrophages express MHC class II and costimulatory molecules and phagocytose myelin \(^{349}\), which suggests a major role for these cells in the presentation of myelin antigens to CD4+ autoreactive T cells \(^{350}\).

All studies performed so far using human and rodent brain, have shown that Mg are able to take up, process and present protein antigen to naïve, memory and
differentiated T cells, leading to stimulation of either T cell proliferation, effector functions (cytokine secretion) or both. Although cultured Mg from developing brain exhibit a more activated phenotype and act as more efficient APC than Mg isolated from the adult CNS, it has been demonstrated that adult Mg activated in vivo effectively induced T cell effector function. In the hierarchy of APC, Mg appear to be as efficient as dendritic cells and are more efficient than B cells in the restimulation of effector T cells but less potent than dendritic cells in inducing primary T cell responses.

In another study, Ford et al., (1996), have shown that Mg isolated from brain/spinal cord rats undergoing graft versus host disease (GVHD), a condition associated with CNS invasion by a limited number of T cell blasts exhibit an activated phenotype, which is indicated by elevated expression of CD45 and MHC class II and also phagocytic activity, appears to be induced by direct interactions with CNS-infiltrating T cells. When tested, ex-vivo GVHD-derived Mg were able to present antigen CD4+ T cells leading to TNF-α and IFN-γ secretion, but failed to support T cell proliferation and induced some degree of T cell apoptosis. These results may represent one of the regulatory mechanisms allowing rapid termination of intracerebral T cell responses.

The T cell stimulatory potential of Mg in vitro, as well as their expression of MHC class II and adhesion/costimulatory molecules are greatly enhanced following myelin phagocytosis, exposure to IFN-γ and/or Granulocyte macrophage-colony stimulating factor (GM-CSF), ligation of CD40, and direct interactions with Th1 cells, suggesting that tissue damage, cytokine milieu and Th cell polarization are all critical factors promoting Mg APC function.

Recently, Pereira et al., (2003) have demonstrated that CAP37 (cationic antimicrobial protein of molecular weight 37kDa), is a chemoattractant for Mg, and that CAP37-treated Mg express class II MHC antigens and produce proinflammatory cytokines and chemokines. CAP37 to known to be a potent activator of monocyte function and that it has the potential to serve as a neuroinflammatory molecule.
REGULATION OF Th1 / Th2 RESPONSES

The T helper (Th) subsets, Th1 and Th2, differ in their effector function and profile of secreted cytokines and has added new insights into pathological immune responses. Upon activation Th1 cells produce IL-2, IFN-γ, TNF-α and TNF-β whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13 predominantly. Th1 cells functions to elicit phagocyte mediated defense against infections and promotes differentiation of CD8+ T cells into active cytotoxic cells whereas Th2 cells mediated humoral immune responses by stimulating the differentiation of B cells and antibody production and regulate Th1 responses through secretion of anti-inflammatory cytokines like IL-4, IL-10.

Th1 mediated immune responses have been implicated in host defense against neurotrophic pathogens as well as in the pathogenesis of EAE and MS. These cells have essential role in activating Mg cells by secreting IFN-γ and TNF-α. Th2 cells, conversely, by their ability to downregulate Mg activation and induction of Th1 responses, inhibit CNS inflammation and suppress EAE. Because of the critical role of the balance of intracerebrally produced Th1 and Th2 cytokines, in CNS environment their regulation is essential and this could occur at various steps after initiation of CNS-specific immune responses:

1. During T cell recruitment to the CNS, as both Th1 and Th2 cells responds to different chemokines like MIP-1α, MIP-1β, IP-10, RANTES and eotaxin and MDC respectively.

2. During intracerebral T cell reactivation.

IL-12 and IL-4 respectively play decisive roles in the development of Th1 and Th2 cells. While at present there is no evidence that resident CNS cells produce IL-4, Mg appears to be a major source of IL-12 during CNS inflammation. In cultured Mg IL-12 synthesis and secretion is induced by IFN-γ / LPS and during antigen specific interaction with Th1 cells. These findings raise the possibility that Mg derived IL-12 may contribute to Th1 skewing within the CNS and hence to the pathogenesis of Th1 mediated CNS disorders. Interestingly, Th1
cells and to a lesser extent Th2 cells, also stimulate Mg to produce high amounts of PGE\textsubscript{2} a potent Th1 down-regulator\textsuperscript{292}, suggesting that during antigen presentation negative feedback mechanisms are induced which may present the Th1 development of newly recruited Th1 cell and stop inflammation\textsuperscript{5}. IFN-\gamma pretreated Mg efficiently restimulates Th1 and Th2 responses both in the presence of relevant protein and antigenic peptide whereas astrocytes only present antigenic peptides to Th2 cells\textsuperscript{290,292}. Krakowsky et al., (1997)\textsuperscript{367} showed differential restimulation of Th1 cell by adult mouse Mg activated with IFN\gamma/ LPS. In contrast, Ma et al., (1999)\textsuperscript{369}, reported that in neonatal mouse Mg, in the absence of any activating treatment, were only able to stimulate T cells towards the Th2 pathway. The workers have further proposed that Mg exposed to TGF-\beta as well as Mg-derived TGF-\beta may play a role in inducing Th2 responses and therefore in the establishment of an anti-inflammatory milieu\textsuperscript{369}. Mg cells are therefore highly versatile cells capable to amplify, downregulate or shape the pro-and anti-inflammatory nature of cellular immunity depending on the environmental context.

Upon antigen-specific interaction with Th1 but not Th2 cells, Mg strongly upregulate the surface expression of MHC class II, CD40 and CD54 molecules\textsuperscript{370}, CD80, CD86\textsuperscript{371} and increased cytokine/chemokine secretion (TNF-\alpha, IL-6 and CXCL10/IP-10)\textsuperscript{371}. Acutely isolated adult Mg stimulated Th1 cells to secrete IFN-\gamma and to a lesser extent IL-2, but were inefficient stimulators of IL-4 secretion by Th2 cells\textsuperscript{336}.

Therefore, the soluble molecules secreted by Th1 and not Th2 cells that infiltrate the CNS can stimulate resident Mg to acquire enhanced effector and accessory cell functions; the Th1-induced effects were not downregulated by Th2 supernatant-mediated bystander suppression\textsuperscript{371}.
MICROGLIA AND ITS ROLE IN ESCAPE OF GLIOMA CELLS FROM IMMUNE SURVEILLANCE:

Comparative study have shown that rat Mg can present myelin basic protein and S100β CNS proteins less efficiently than thymocytes and appear to present C6 glioma cells to cytotoxic T lymphocytes. The observation suggests that the defense function of Mg cells against C6 glioma is severely compromised and the observed deficiency in antigen presentation may play a role for malignant brain tumor to grow in vivo. Tran et al., (1998), have shown that Mg in astrocytic glioma are well equipped to function as APC, however, neoplastic astroglia appear to acquire the capacity to downregulate Mg MHC class II expression and at the same time, may induce T cell clonal anergy through aberrant expression of MHC class-II molecules. Because the Fas pathway has been proposed to play a role in immune evasion, and it has been suggested that Mg are a major source of Fas L expression in brain tumors and possibly contribute to the local immuno-suppressive milieu of malignant gliomas.

MICROGLIA AND TUMOR REJECTION:

One of the morphologic hallmarks of gliomas is inflammatory infiltrates with accumulation of macrophages/microglial cells and T lymphocytes (both CD4+ and CD8+) at the tumor site. Despite the high numbers of macrophages in brain tumor (gliomas being extensively studied), often more than 50% of total tumor associated cells, hardly any tumor necrosis is detectable. So, the question arises that what are the signals leading to the intense inflammatory response to the CNS tumors and what are the factors leading to the cellular immunosuppression?

Two novel chemotactic factors for monocytes have been identified in gliomas & sarcomas, termed as glioma-derived chemotactic factor (GDCF)-1 and -2. GDCF-1 is identical to MCP-1. Glioblastoma cells also produce the immunosuppressive molecule transforming growth factor-β2 (TGF-β2), a chemoattractant for monocytes. Growth factors for macrophages, which are
produced locally either by the tumor cells, or by adjacent reactive astrocytes or by activated T cells having infiltrated the tumor mass may lead to expansion of microglial cells, by secreting granulocyte macrophage-colony stimulating factor (GM-CSF). Frei et al., (1987) have shown that Mg cells respond to GM-CSF by increased growth. GM-CSF further stimulates a broad range of functional activities of monocytes/macrophages including their tumoricidal killing, accessory cell function, phagocytosis and oxidative metabolism. In vitro, Mg cells exposed to IFN-γ and/or GM-CSF have been found to become cytotoxic for glioblastoma cells.

In vitro, not only astrocytoma but also glioblastoma cell lines secrete GM-CSF when stimulated with IL-1 or TNF-α; while no evidence exists in vivo studies. Unlike GM-CSF, TGF-β2 mRNA is expressed in ex vivo tested glioblastoma tissues. Absence of GM-CSF in vivo may be explained by the presence of tumor-derived inhibitory factors such as TGF-β2 and PGE, which suppress GM-CSF production by glioblastoma cells in vitro.

The intrathecal production of IL-10 may be fundamental for the host response to tumors and to infections since IL-10 induces suppression of production of reactive oxygen intermediates (ROI) and nitric oxide (NO), molecules involved in the killing of tumor cells. Since IL-10 inhibits the synthesis of cytokines by activated monocytes/macrophages, the observed expression of IL-10 at later stages of meningeal inflammation may reflect a regulatory circuit, which counteracts the inflammatory process maintained by ongoing production of cytokines. This view is further supported by the findings that IL-10 is produced in the nervous system of mice having recovered from experimental autoimmune encephalomyelitis (EAE) rather than during acute disease. The latter is characterized by activation of IL-1, IL-2, IL-4, IL-6 and IFN-γ genes. Interestingly, deactivation of macrophages is not only achieved by soluble factors but also by tumor extracellular matrices.

Taken together, tumor-associated macrophages/microglia cells have a complex relationship with the neoplastic cells of the tumors. On one hand, they exhibit symbiotic relationship by production of growth factors/inhibitors, on the other hand microglia can be activated to inhibit tumor growth and destroy tumor
cells. The outcome depends on the sum of individual functions. In glioblastomas, the imbalance leads to an ‘anti-immune network milieu’ which enables the tumor cells to avoid immune recognition and rejection.

BIOLOGICAL PROPERTIES OF MICROGLIA

1) MICROGLIAL TURNOVER :

Tissue macrophages are terminally differentiated cells derived from circulating monocytes and they are replenished by slow turnover of the resident cell population by new monocytes. In case of Mg turnover, very few studies have been performed. Studies of mouse chimeras created by bone marrow transplantation into lethally irradiated animals have provided evidence that perivascular macrophages undergo repaid turnover, but ramified parenchyma Mg turnover is very slow even when followed for prolonged survival times 91,195. The marker used in these studies was a histocompatibility antigen which has been criticized as a potential downregulator in ramified Mg. Further studies using a constitutively expressed lysosomal enzyme, gluco-cerebrosidase, however, concluded that upto 20% of the ramified perivascular Mg can be repopulated throughout the lifetime of the animal 383. Thus, the great majority of ramified cells turnover very slowly or never in the lifetime of the mature animal.

2) PROLIFERATIVE POTENTIAL OF Mg IN SITU :

If ramified Mg are not repopulated (or repopulated very slowly), it is reasonable to assume that they may be repopulated by local proliferation. In a study on normal mice, mitotic activity was measured by determining the amount of H1-thymidine incorporation; less than 0.05% of ramified Mg was labeled in one hour384. The results indicate that intrinsic Mg has limited proliferative potential in the normal state. In contrast, Mg cells have a higher proliferative potential in
response to a wide variety of diseases\textsuperscript{385}, including injuries like peripheral nerve injuries\textsuperscript{386, 387} limited excitotoxic\textsuperscript{388}, or hypoxic brain injury\textsuperscript{389} and changes in the terminal fields of neurons undergoing degeneration, such as the entorhinal cortex / perforant pathway model\textsuperscript{390}. In a proliferating cell nuclear antigen (PCNA) labeling study of AIDS brains, an unusually high rate of PCNA labeling was detected, including cells colabeled with RCA-1 (Morris 94). These results indicate that Mg cells turnover more rapidly in AIDS and probably other CNS disorders\textsuperscript{141}.

3) \textit{PROLIFERATIVE POTENTIAL OF Mg IN VITRO}:

\textit{In vitro} studies of Mg provided conflicting results with respect to proliferative potential of Mg and may be in part related to maturity of the microglial cell. Mg cells from adult brain tissue (temporal lobectomy specimens) indicated little proliferative potential in response to a panel of growth factors\textsuperscript{158}. Human foetal Mg, however, proliferate \textit{in vitro} given the proper conditions. In a study of human foetal Mg (20-24 weeks gestational age), Mg had microscopic features and biological properties consistent with amoeboid Mg\textsuperscript{392}. These cells also proliferate in response to recombinant human granulocyte-monocyte colony stimulating factor (GM-CSF) and to some extent M-CSF\textsuperscript{393}. Mg when cultured with recombinant M-CSF, show downregulated HLA-DR\textsuperscript{394}. In contrast, Mg from adult autopsy brains had limited proliferative potential in response to M-CSF, but better response to GM-CSF. It may be due to the exposure of adult Mg to a number of factors that are not present in foetal brain and that have been shown to activate Mg\textsuperscript{395}. In animals also Mg proliferative potential is firmly established\textsuperscript{396-398}.

4) \textit{APOPTOSIS AND CONTROL OF MICROGLIA CELL NUMBER}:

The activation of microglia (Mg) in apoptosis\textsuperscript{399}, is a double-edged response; sometimes they act as scavengers removing tissue debris and inducing apoptosis in damaged cells, and in more subtle injury they exert a surveillance function and might
play a protective role. Resting Mg is resistant to Fas ligand (Fas L) treatment; induction of Fas L-mediated apoptosis was achieved by treatment with IFN-γ and TNF-α. Upregulation of Fas expression, and downregulation of BCL-2 and BCL-XL but not Bax paralleled the treatment of these cytokines. Fas/Apo-1 is a member of TNF-receptor and signals apoptotic cell death in susceptible target cells. Activation of Mg by TNF-α and IFN-γ was also accompanied by increased amount of mRNA for the apoptosis inhibitor FLIP, an effect that does not protect the cells from Fas L-induced apoptosis. This Fas L-mediated pathway in Mg involves reactive oxygen intermediates because the anti-oxidant N-acetylcysteine and glutathione interfere with induction of apoptosis. Surprisingly, Mg constitutively expresses Fas L on the cell surface and possibly contributes to the local immunosuppressive milieu of malignant gliomas. However, blocking of endogenous Fas-Fas L interaction with Fas-Fc fusion protein does not enhance the survival of Mg, excluding the possibility of suicide of fratricide mechanisms. By this expression of Fas L and their TNF-α/IFN-γ dependent sensitivity, Mg cells may influence the course of T-cells mediated diseases of the CNS.

Fas expression is, in fact, regulated at the level of mRNA expression; TNF-α and IFN-γ induced Fas mRNA by approximately 20 fold; STAT-1α and NF-κB activation are involved in IFN-γ or TNF-α mediated Fas upregulation in Mg, respectively. The cytokine Tumor growth factor-beta (TGF-β) inhibits basal expression of Fas as well as cytokine mediated Fas-expression by Mg. Upon incubation of Mg cells with Fas L-expressing cells, approximately 20% of the cells underwent Fas-mediated cell death, which increased to 60% when cells were pretreated either with TNF-α or IFN-γ. TGF-β treatment inhibited Fas-mediated cell death of TNF-α or IFN-γ stimulated Mg cells. Fractalkine also inhibits Fas-mediated apoptosis of Mg, suggesting modulation of the apoptotic process by the cytokine milieu.

Reid et al., (1993), have demonstrated that in rodents proliferating Mg are also susceptible to apoptotic cell death. The presence of simultaneous apoptosis and proliferation of Mg may seem counter intuitive, but apoptosis may be a means of controlling Mg cell numbers during reactive conditions. A physiologic role of
apoptosis has been invoked to explain control of cell number in development\textsuperscript{408}, and other disease states, including resolution of acute inflammation\textsuperscript{409}. It may be a mechanism for maintaining cell numbers at a critical set point determined by a complex interaction of cellular and extracellular factors, including growth factors and cytokines\textsuperscript{410}. The knowledge about its role in reactive cellular changes in the brain is still in its infancy. Dalmau et al., (2003)\textsuperscript{136}, have reported that in developing rat brain, Mg apoptosis occur only locally at certain developmental stages.

Of particular interest is the fact that Vitronectin, a beta3 integrin (CD61) detected on Mg cell, is the putative receptor for apoptotic cellular debris (apoptotic bodies')\textsuperscript{411, 412}. Vitronectin is an adhesive type of extracellular protein similar to fibronectin and laminin\textsuperscript{413}.

Lui et al., (2001)\textsuperscript{414}, have reported that over-activation of Mg with higher concentrations of lipopolysaccharide (LPS>1 ng/ml) resulted in a time-and dose-dependent apoptotic death of Mg as defined by DNA strand breaks, surface expression of apoptosis-specific markers (phosphatidylserine) and activation of Caspase-3. In contrast, astrocytes were insensitive to LPS-mediated cytotoxicity.

A novel microglial gene, granule cell death-10 (gcd-10) is expressed in Mg and upregulated in an early period of granule cell death. This gene is involved in the dynamics of lysosomal membranes associated with Mg activation both in vitro and in vivo\textsuperscript{415}.

Brough et al., (2002)\textsuperscript{416}, have reported the role of purinergic receptor (P2X7) in inducing cell death in Mg cells.

While production of anti-inflammatory mediators may prevent further recruitment and activation of immune cells, apoptotic cell death mediated by receptors of the TNF-R superfamily represents the major mechanism to eliminate effector cells\textsuperscript{417,418}.

5) \textbf{PHAGOCYTIC ACTIVITY OF MICROGLIA}:

Cells of the mononuclear phagocyte system are professional phagocytes, possessing a number of specific receptor molecules that facilitate this function, including receptors for the immunoglobulin (FcR), complement (CR3) and glycation
endproducts. Resting microglia (Mg) has been demonstrated to possess almost all these receptors. Fc and complement receptors mediate target phagocytosis by Mg in all neuroinflammatory conditions associated with specific humoral responses against CNS pathogens. Both morphologic and functional evidence suggests that Mg have phagocytic properties. Further presence of cytoplasmic vacuoles and lysosomal bodies in Mg, suggests their phagocytic role. The uptake of apoptotic cells by Mg suggested that this uptake is tolerogenic and results in a reduced proinflammatory cytokine production and a reduced activation of encephalitogenic T-cells, which may help to restrict an autoimmune inflammation and minimize damage to the damaged brain.

In rat, ultrastructural lectin binding studies have demonstrated that ramified Mg phagocytoses synaptic terminals of axotomized neurons. In several other studies, amoeboid Mg has been reported to ingest foreign objects (latex beads), immunoglobulin-coated erythrocytes, and dead cell. Lee et al., (1994) have also reported the role of Mg in phagocytic removal of bacteria and fungi. The mechanism is possibly coupled to a respiratory burst and generation of reactive oxygen intermediates, which are one of the means for cell killing. Macrophage colony stimulating factor (M-CSF) increased Mg cell phagocytic activity and could be important in promoting microglial clearance of abnormal protein aggregates such as amyloid beta plaques.

Ramified Mg are evidenced to have only a limited phagocytic potential; whereas, amoeboid Mg and blood-derived monocytes are the major phagocytes in the CNS. The type of cell that contributes to phagocytosis in the CNS is the reflection of the nature and extent of tissue damage. When the degree of tissue damage is limited to selective to individual cells, ramified Mg and perivascular Mg appear to be the major phagocytes, but when the damage is more massive, intrinsic Mg are assisted by fresh influx of circulating blood monocytes. This is known as the 'graded response' in Mg cell.

It seems increasingly likely that ramified Mg become phagocytic as they revert to a morphologically less differentiated state, namely the amoeboid Mg or transitional forms, such as rod cells or lamellae cells.
Another supportive feature in this perspective is the involvement of Mg in limited phagocytosis of individual synaptic termini, probably leading to synaptic remodeling. In addition to the detection of apparently displaced synaptic termini and their engulfment, ramified cells undergo proliferation that can be blocked by Adriamycin treatment.

Phagocytic Mg cells are independent from the CNS cytokine network in their transition from a resting to an activated phenotype; and different cellular substrates, regardless whether they are of neuronal, glial or even malignant origin, result in similar morphological and functional changes.

MICROGLIAL SECFRETORY PRODUCTS

i) NITRIC OXIDE (NO)

Microglia (Mg) cells are known to produce reactive oxygen species including superoxide anion and nitric oxide. Cells with constitutive expression of nitric oxide synthase (NOS) produce small, short-lived pulses of NO. Neuronal derived short bursts of NO have been implicated in neurotransmission. In contrast, cells with inducible NOS (iNOS), produce high and sustained levels of NO that may be toxic. Cells with iNOS have been shown to mediate toxic effects on microbes with NO. Furthermore, cultured human Mg has only limited low-level production of NO in response to stimuli including viral infections and cytokines. Nitrites are readily detected in human astrocytes. Since, Mg play a major role in regulating astrocyte functions, the NO-mediated events often ascribed to Mg may be indirect through their effects on astrocytes, at least in the human.

Prothrombin, a zymogen of thrombin induces NO release and mRNA expression of iNOS in rat brain Mg. Serum also enhances LPS induced production of NO. Hoffmann et al. (2003), reported that calcium ion (Ca²⁺) concentration is essential for release of NO by Mg cell. On the contrary, apolipoprotein E (ApoE) suppresses release of NO by activated Mg cells in a dose-dependent manner.
ii) **OXYRADICALS**

While it is clear that oxyradical molecules can be extremely toxic to surrounding cells, the regulation of Mg oxyradical production is relatively unknown. The factors that regulate oxyradical production by Mg possibly are factors, which directly initiate superoxide anion release via activation of the NADPH oxidase, and those factors that modulate superoxide anion release by acting synergistically with inducing agents. Using cytochrome C reduction assay, Colton et al., (1992)\(^440\) have shown that opsonized zymosan A23187 (a Ca\(^{2+}\) ionophore) phorbol esters (PMA) and IL-1\(\alpha\) induce measurable superoxide anion production. Pretreatment of the Mg cells with Interferon-alpha (IFN-\(\alpha\)) and IFN-\(\beta\) enhances their production\(^440\). Other potentiating agents include growth hormone and high extracellular potassium levels in the media. In contrast, nor-epinephrine reduces the potentiation of Mg superoxide anion production by IFN-\(\alpha/\beta\)\(^440\).

The effect of Mg-generated oxyradicals in CNS is not clearly described. They may play an important role in the neuropathology of various disease states\(^180\). For example, amyloid deposits (plaque formation by aggregation of beta A4) in Alzheimer’s disease. CD36, a class B scavenger receptor has a role in fibrillar \(\beta\)-amyloid-induced \(\text{H}_2\text{O}_2\) production by Mg, and imply that CD36 can mediate binding to fibrillar \(\beta\)-amyloid\(^441\).

iii) **QUINOLINIC ACID**

Another potential neurotoxin secreted by Mg is quinolinic acid, which acts through the glutamate receptor of neurons to produce excitotoxic cell injury\(^442\). Brain macrophages are the major cellular source for quinolinic acid, since they are the only cells to contain the rate-limiting synthetic enzyme indoleamine-2, 3-dioxygenase \(^443\). IFN-\(\gamma\) has an upregulatory effect on the production of this acid and is elevated in a number of inflammatory disease states\(^443\). This acid has been implicated as a potential excitotoxin in neurodegenerative disease, such as AIDS dementia\(^444\).
iv) **PLATELET ACTIVATING FACTOR (PAF)**

PAF is a phospholipid-derived inflammatory mediator. It is a chemically defined small molecule, acetyl glycerol ether phosphocholine. It is produced by several cell types in CNS injury and has been implicated as a potential neurotoxin in brain trauma, ischemia and viral infections, particularly AIDS. Direct demonstration of PAF production from human Mg waits to be reported.

v) **COMPLEMENT PROTEINS**

Refer to “Fc and Complement receptors”

vi) **PROTEASES**

Among the various enzyme molecules that are derived from Mg, interest has been focused on matrix metalloproteinases, or collagenases. Cultured Mg also secretes various proteases such as elastase, urokinase-type plasminogen activator (uPA) and plasminogen.

Mononuclear phagocytes as part of an inflammatory reaction and which digest extracellular matrix molecules release matrix metalloproteinases. Evidences suggest that Mg is immunoreactive for matrix metalloproteinase suggesting that this protease may be relevant to disease processes. In normal brain, low levels of matrix metalloproteinase immunoreactivity were detected, which increased in disease states such as AD. Since extracellular matrix molecules are detected in the CNS in synaptic clefts and associated with blood vessels, it is tempting to speculate that metalloproteinase may be involved in the synaptic remodeling or synaptic stripping or functional attributes of perivascular Mg.

Among the other proteases, plasminogen has neurotrophic activity like promotion of neurite outgrowth. Most interestingly, it significantly increased dopamine uptake and the number of tyrosine hydroxylase-positive neurons in mesencephalic culture. These results suggest that plasminogen enhances the survival and/or maturation of various types of neurons. Further, the results leads to the possibility that plasminogen exerts the neurotrophic effects through the binding to the receptor-like molecules on the neuronal surface.
NEURO-ONCOLOGY

Central Nervous System (CNS) cancer remains a devastating disease, but after more than 30 years of intensive research, insights into key questions in neuro-oncology have yielded, and these insights are being translated into better and more successful care of patients with primary and metastatic nervous system cancer. About half of the brain tumors are metastatic foci of tumors originating outside the CNS, while the other half are primary tumors of central nervous tissue. Any of the cell type that comprise the nervous system may give rise to tumors, but the most common primary nervous system tumors arise from the glial cell series. Some tumors may not actively invade the brain, i.e. benign tumor, but may cause morbidity and mortality due to raised intracranial pressure or by interfering with the vital brain areas.

Classification and Grading of Brain Tumors:

The World Health Organization (WHO) has classified primary CNS tumors into nine different categories based on the presumed cell of origin (Table 1). The tumors range in degree of aggressiveness from ‘low grade’ to ‘high grade’ tumors (Table 2). WHO grades must be considered as scale of malignancy reflecting the biological behavior and thus the ‘average’ clinical prognosis of all tumor entities of the same grade.

Low-grade tumors of the brain represent a large proportion of primary brain tumors, ranging from 15% to 35% in most reported case. They include a remarkable diversity of lesions all of which have been lumped together under the heading of ‘low-grade glioma’ (Table 3). High-grade tumors such as anaplastic astrocytoma and GBM account for the majority of astrocytic tumors and are generally referred to as malignant glioma.

In the WHO classification, ‘low-grade’ (slow growing) tumors are classified either as grade I or grade II, depending on whether they are circumscribed or not. Grade I tumors are circumscribed and exhibit moderate cellularity and thus
have a better prognosis than grade II tumors, because of the latter's poorly defined margins or diffuse spread (erroneously referred to as 'infiltrative') often preclude complete surgical resection. 'High-grade' tumors are characterized by rapid growth, either in anaplastic foci that develop in a low-grade tumor, or in a large portion or the entire volume of the tumor mass. The appearance of anaplastic foci in a pre-existent grade I or II tumor results in classification as a grade III tumor. Marked sizes of anaplasia in a large portion of the tumor or in entire mass leads to classification as grade IV. Clinical experience has shown that grade III tumors progress less rapidly than grade IV tumors.

| World Health Organization Categories of Primary Central Nervous System Tumors |
|---------------------------------|-----------------|
| Type of Tumor                  | Frequency (%)   |
| Infiltrative astrocytoma        | 42.4            |
| GBM                             | 40.6            |
| Medulloblastoma                 | 3.6             |
| Oligodendrogloma                | 3.5             |
| Ependymoma                      | 3.0             |
| Meningioma                      | 2.0             |
| Mixed Oligoastrocytoma          | 1.7             |
| Pilocytic Astrocytoma           | 1.5             |
| Others                          | 1.7             |

Table 1:
Adapted from Polednak et al., Cancer 95: 330, 1995 (Suppl).
Grading System Description

<table>
<thead>
<tr>
<th>Grading System</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Kernohan and Sayer</td>
<td>Four-tiered system, with grade 4 the malignant.</td>
</tr>
<tr>
<td>WHO</td>
<td>Three-tiered system with grade 1 as well differentiated, astrocytoma, grade 2 as AA, grade 3 as GBM.</td>
</tr>
<tr>
<td>Daumas-Duport</td>
<td>Four-tiered system based on the presence or absence of 4 major criteria (nuclear atypia, mitosis, endothelial proliferation and necrosis), with Grade 1 having none of these features, Grade 2 having one, Grade 3 having two and Grade 4 having at least three features.</td>
</tr>
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Table 2:—

Source: Seminars in Oncology, 27 No.6 (Suppl) 2000.

**Neuroepithelial Tumors of the CNS:**

1. Astrocytic tumors [glial tumors—categories I-V, below—may also be subclassified as invasive or non-invasive, although this is not formally part of the WHO system, the non-invasive tumor types are indicated below. Categories in italics are also not recognized by the new WHO classification system, but are in common use.] 464,466
   1. Astrocytoma (WHO grade II)
      1. variants: protoplasmic, gemistocytic, fibrillary, mixed
   2. Anaplastic (malignant) astrocytoma (WHO grade III)
      1. hemispheric
      2. diencephalic
      3. optic
Review of Literature

4. brain stem
5. cerebellar

3. Glioblastoma multiforme (WHO grade IV)
   1. variants: giant cell glioblastoma, gliosarcoma

4. Pilocytic astrocytoma [non-invasive, WHO grade I]
   1. hemispheric
   2. diencephalic
   3. optic
   4. brain stem
   5. cerebellar

5. Subependymal giant cell astrocytoma [non-invasive, WHO grade I]

6. Pleomorphic xanthoastrocytoma [non-invasive, WHO grade I]

2. Oligodendroglial tumors
   1. Oligodendroglioma (WHO grade II)
   2. Anaplastic (malignant) oligodendroglioma (WHO grade III)

3. Ependymal cell tumors
   1. Ependymoma (WHO grade II)
      1. variants: cellular, papillary, epithelial, clear cell, mixed
   2. Anaplastic ependymoma (WHO grade III)
   3. Myxopapillary ependymoma
   4. Subependymoma (WHO grade I)

4. Mixed gliomas
   1. Mixed oligoastrocytoma (WHO grade II)
   2. Anaplastic (malignant) oligoastrocytoma (WHO grade III)
   3. Others (e.g. ependymo-astrocytomas)

5. Neuroepithelial tumors of uncertain origin
   1. Polar spongioblastoma (WHO grade IV)
   2. Astroblastoma (WHO grade IV)
   3. Gliomatosis cerebri (WHO grade IV)

6. Tumors of the choroid plexus
   1. Choroid plexus papilloma
2. Choroid plexus carcinoma (anaplastic choroid plexus papilloma)

7. Neuronal and mixed neuronal-glial tumors
   1. Gangliocytoma
   2. Dysplastic gangliocytoma of cerebellum (Lhermitte-Duclos)
   3. Ganglioglioma
   4. Anaplastic (malignant) ganglioglioma
   5. Desmoplastic infantile ganglioglioma
      1. desmoplastic infantile astrocytoma
   6. Central neurocytoma
   7. Dysembryoplastic neuroepithelial tumor
   8. Olfactory neuroblastoma (esthesioneuroblastoma)
      1. variant: olfactory neuroepithelioma

8. Pineal Parenchyma Tumors
   1. Pineocytoma
   2. Pineoblastoma
   3. Mixed pineocytoma/pineoblastoma

9. Tumors with neuroblastic or glioblastic elements
   (embryonal tumors)
   1. Medulloepithelioma
   2. Primitive neuroectodermal tumors with multipotent differentiation
      1. medulloblastoma
         1. variants: medullomyoblastoma, melanocytic
            medulloblastoma, desmoplastic medulloblastoma
      2. cerebral primitive neuroectodermal tumor
   3. Neuroblastoma
      1. variant: ganglioneuroblastoma
   4. Retinoblastoma
   5. Ependymoblastoma
Epidemiology:

Proper registration of a population based data on cancer pattern in a particular region is important for determining the etiology, risk factors, vulnerable patches in demographic contour of the community and priority zones to control the disease.

Although primary brain tumor represents only 2% of the estimated new cases of cancers occurring in adults, it is among the most lethal and difficult to treat forms of cancer. A cancer based epidemiological study sponsored by Department of Science and Technology, West Bengal, India, was undertaken by our laboratory and furnished the following information 467.

A total number of 36,509 cancer patients were registered in Kolkata during the period of 3 years, of which 2.39% cases were found to be malignant brain tumor 467, 468. The incidence of all adult primary brain tumors, including benign and malignant tumors is 11.8 per 1,00,000 person per year, with malignant brain varies between 2-3% throughout the world 469. When intracranial malignancies were considered in respect to neurological disorders, they showed 7.59% incidence. They were primary tumors and broadly divided into two major categories depending on their origin i.e. tumors of glial origin, namely glioma and tumors of non-glial origin. Gliomas were the most prevalent type occupying 60.03% of the total spectrum of neoplasm in brain while non-glial tumors occupying 39.97% of the cases. Among gliomas, the incidence of astrocytoma was most frequent occupying 58.89% of glioma incidences 470. As Glioblastoma multiforme (GBM) is now regarded as high grade of astrocytic tumors 471, their incidences if considered as astrocytoma, will reach upto 71.52%. Again mixed glioma, reported 15.42% among the glial tumors, also have abnormal astrocytic cells. Therefore very high incidence of astrocytoma among the glioma patients showed similarity with the world scenario (over 80% are astrocytic tumors among gliomas) 472. Oligodendroglioma (7.92%) and ependymoma (5.14%) were the other two-glioma subtypes. Non-glial tumors consist of many different varieties of which, the incidences of meningiomas were most frequent (30.96%) followed by craniopharyngioma 473.
The occurrence of total brain tumor in male was found to be comprehensively high with a male to female ratio of 2.15. For glial and non-glial tumors the ratios were 2.7 and 1.57 respectively. Noticeably, higher incidences of male glioma patients varied widely from the global trend where the ratio was 1.6 with some deviation in local communities \(^{469}\). Even all subtypes of glioma showed more than two fold incidences of men than women. Except meningiomas, pineal body tumor and neuroblastoma, all other brain tumor types were found to be dominated by males.

When age specific distribution of the incidence of intracranial malignancy were considered, a more or less similar trend in both male and female as well as for total brain tumor patients was found. However, two major deviation of the trend was observed between male and female population. Between the age group of 20-29 years, a comparatively low incidence of brain tumor was observed in females. It was then increased to a considerable degree among 40-49 years of ages than their male counterparts. Again, 50-59 years of age group also contain significant female brain tumor patients. On the contrary the male patients showed much regularity in their incidence curve of the disease. However, from 60 years of age, a sharp decline of the hospital patients was observed for both sexes \(^{474,475}\). The highest incidence of brain tumor around 45 years of age may be due to estrogen deficiency during menopause in females\(^ {476}\). The general tendency of higher incidence of malignant brain tumor in age group of 30-60 years for both sexes might be correlated to higher exposure to environmental carcinogens. These make certain susceptible individuals more prone to transformation of proto-oncogene to oncogene and also due to increased rate of mutational changes from the middle age groups \(^ {477}\).

Descriptive epidemiological studies have shown some geographical variations in the incidence of brain tumors, showing general tendency of that to be highest in developed, industrial countries such as in Western Europe, North America and Australia \(^ {478}\). In multiracial countries, Caucasians are frequently more affected than people of African or Asian descent \(^ {457,479}\). Preferential manifestation in specific age groups is a hallmark of CNS tumors and often yields together with tumor size. Peak incidences are found in children and in adults aged 45 to 70 years. There is
general tendency that gliomas and embryonic tumors occur more frequently in males while meningiomas occur most frequently in females.

AETIOLOGY OF BRAIN TUMORS:

The etiology of brain tumors in human is still not very clear. Although, during the past decade numerous analytical epidemiological studies have been performed, the associations with environment, diet and life style factors have shown either not being statistically significant or found to be inconsistent. Analytical epidemiological studies have shown an increased risk of brain tumor development in association with certain occupations; for example in farmers, dentists, firefighters, metal workers and in rubber industries. But attempts to identify the specific causative agents have been unsuccessful. In a recent study, identified in coal miners of Australia, which was almost five times more than Australian general population. Occupational exposure to organic solvents also has been linked to the cluster of brain tumors, however confounding factors could not be excluded. Increased incidence of CNS neoplasms in anatomists, pathologists and embalmers, hints at a possible role of formaldehyde but it has emerged that in an industrial setting exposure to this carcinogen has been found not to be associated with an increased risk. Several studies have accounted the role of herbicides, fungicides and their derivatives; however, the findings has been questionable studies have also shown a slightly increased risk for white collar workers, managers and people with higher socio-economic status, but this trend has not being consistent. Electrical and electronic work has been found to have a weak association with brain tumors. Wertheimer et al., (1979) have shown that residential electromagnetic field exposure may lead to higher incidence of brain tumors, especially in children. Electromagnetic fields generated by electric blankets were reported to increased brain tumor risk in children, though recent study does not confirm this. However, Villeneuve et al., (2002) have reported that occupational magnetic field exposure increases the risk of glioblastoma multiforme, especially in men; while no association
of the exposure with astrocytoma and other brain tumors was found. Therapeutic X-irradiation has been implicated as the sole environmental factor unequivocally associated with an elevated brain tumor risk; though the role of diagnostic X-ray application remains unclear. Radiation induced meningiomas and also gliomas have been observed with low dose irradiation for tinea-capitis. Children receiving prophylactic CNS irradiation for acute lymphocytic leukemia appear at a high risk for the development of low and high-grade astrocytomas and glioblastomas. Irradiation of carniopharyngioma, pinealoma, germinoma, and also brachytherapy of malignant gliomas, have been reported to generate second primary tumors of neuroepithelial origin. Smoking has been shown to have no significant association with brain malignancy; as also no good evidence linking the increasing incidence of brain tumors to mobile phones was found.

Nitrosocompounds play a role in the development of brain tumor since some of the agents have been found to be neuro-carcinogens in rodents. Nitrosocompounds have been detected in nitrite preserved food and in beer but they can also be found in the stomach following uptake of their chemical precursors. Analytical studies suggest that glioma risk may be somewhat higher in people with a high intake of meat, particularly cooked ham, processed pork and bacon. Dietary habits during pregnancy have also been suggestive of an adverse of food likely to be a source of nitrosocompounds. Studies have also demonstrated inverse relationship with the intake of fruits, vegetables, and Vitamin C. However, no association has been observed with nitrite in drinking water. Numerous anecdotal reports on the occurrence of gliomas, following a head injury have been observed but a casual relationship could not be established. Epidemiological studies have shown a weak but inconsistent association of both in the adult and in regard to perinatal traumatic head injury.

Several oncogenic viruses are also found to be capable of inducing neuroepithelial and embryonic tumors in rodents. Primitive neuroectodermal tumors have been shown to be induced by SV40 large T, which are morphologically indistinguishable from cerebellar medulloblastomas in humans. However, the identification of SV40 related DNA sequences in childhood brain tumors have
become an elusive goal 521. Studies have also been made to determine the role of infrared exposure to animal viruses; close contact with animals on a firm has shown a weak association in one study in children 522 but not in another. The risk associated with the use of barbiturates has demonstrated inconsistent results both for adults and transplacentally 523. No specific mutations or mutational hot spots have been found in human brain tumors. Further inconsistent epidemiological data corroborate results obtained from the analysis of mutations in sporadic human gliomas. The predominance of GC-AT transition mutation at CpG sites points to an endogenous formation rather than a causation by chemical carcinogens524. New insights into the causes and potential treatment of CNS tumors have come from discovering connections with genes that control cell growth, differentiation, and death during normal development.

So, there is no particular report indicating the possible cause (viral, chemical or traumatic) of brain tumor in humans, although a range of cerebral tumors can be induced in animals experimentally.

**EXPERIMENTAL INDUCTION OF BRAIN TUMORS:**

Several workers have conclusively inducted neural neoplasms using chemical carcinogens in experimental models 525. Polycyclic aromatic hydrocarbons (PAH) were the first carcinogens used to induce brain tumors in experimental animals526. The most effective PAH are 3- and 20-methylcholantherene, ben20(a)pyrine and 7,12-diethylbenz(a)anthracene (DMBA). It has been found that DMBA is the only PAH, which can produce tumors in the offspring following intravenous injection in pregnant rats on the day 15 of gestation 527.

However, the most effective chemical carcinogens that can induce CNS tumors are the alkylating agents. Nitrosourea-derivatives, particularly methyl (MNU) and ethylnitrosourea (ENU) have been implicated for high incidence of brain tumors in rats after systemic administration 528. It has been found that N-N' Ethyl nitrosourea (ENU) is particularly powerful when administered as a single dose transplacentally or shortly after birth 529. The susceptibility of the rat CNS to the
carcinogen has been shown to begin on the 10th prenatal day, and increases gradually and reaches its maximum at birth, when a single dose is approximately fifty times more effective than in adult rats.

Tumors induced by ENU have been diagnosed as oligodendrogliomas, astrocytomas and ependymomas, according to the glial precursor from which they seemed to occur. Burger et al., (1988) demonstrated selective induction of oligodendroglioma with ENU, which indicates that the neoplastic transformation can occur in a differentiated glial cell or a precursor cell committed to oligodendrocytic differentiation; and that transformation of a pluripotent stem cell is not necessary. Recent evidence however, demonstrates that induced ependymomas are actually primitive neuroectodermal tumors with neuronal differentiation.

Several workers had worked out the mechanism of ENU action. Malignant transformation by alkylating agent is the mode of interaction of ENU on cellular DNA. Oxygen centered base alkylation adducts are directly mutagenic and most evidences suggests that the most abundant of these, $O^6 - e^G$ plays a major role in the mutagenesis and carcinogenesis by ENU. The major O-alkylated base is $O^6$- alkylguanine which during DNA replication mispairs with deoxythymidine causing G:C:A:T transition mutation. $O^6$- ethylguanine is repaired by $O^6$- alkylguanine DNA alkyltransferase (AGAT) and this occurs less efficiently in brain. This deficiency of the CNS was considered to be the mechanism of preferential induction of brain tumor by ENU application.

Molecular genetics study in experimentally induced tumors does not have major role of transformation-associated gene in ENU or MNU induced CNS tumors. However, a new oncogene ‘neu’ has been detected by transfection into NIH 3TC cells of ENU induced rat brain. Several oncogenic viruses have also been evidenced to produce brain tumors in experimental models. Rouse sarcoma virus (RSV) were among the firsts viruses found to have neuro-oncogenic activity in animals. Polyoma virus and Papilloma Virus have also been found to develop brain tumors in mice when inoculated at high titre in neonatal mice. SV40, the DNA virus and its transforming
gene, large T, have been reported to exert a broad range of tumorigenic effects in experimental animal models.  

**TUMOR ANTIGENS**

It is now well established that genetic alterations like mutation, gene amplification, chromosomal deletion or translocation lead to the expression of altered protein in tumor cells; which in turn provides antigenic targets for host immune responses. Hundreds of genes have been detected which are preferentially expressed or over-expressed in neoplastic cells. Accordingly, tumor antigens have been categorized into several groups. The MAGE, BAGE, and GAGE families of genes were discovered.  

*Class I HLA-restricted differentiation antigens:*  
These antigens are shared between tumors and the normal tissue from which the tumor arose; most have been found in melanomas and normal melanocytes. Epitopes recognized by both CD8+ and CD4+ T cells can be derived from melanosome proteins.  

*Class I HLA-restricted widely expressed antigens:*  
It is assumed that the many epitopes expressed on normal tissues are below the threshold level for T-cell recognition, while their over-expression in tumor cells can trigger an anti-tumor response by breaking a previously established tolerance. These widely expressed gene products have shown varieties of mechanisms defined epitopes through alterations in gene transcription and translation. Genes encoding widely expressed antigens are CEA, ART-4, Cyp-B.  

*Class I HLA-restricted tumor specified antigens:*  
These antigens are expressed only in the individual tumor where they were identified. Dudley et. al. (1996) have demonstrated that in mouse models unique antigens are more immunogenic than the other groups of shared antigens; and since
these unique antigens are responsible for rejection of tumor transplants, they are also known as tumor-specific-transplantation-antigens (TSTA). Genes encoding these antigens include CDK4\(^{551}\), MUM-1 (melanoma)\(^{552}\), MUM-2 (melanoma)\(^{553}\), catenin (melanoma)\(^{554}\), HLA-A2-R1 701, ELF2 m (lung)\(^{555}\), Caspase-8 (H/N tumors)\(^{556}\) etc.

**Class II-HLA restricted antigens:**

The first epitope presented by a class II HLA and capable of provoking a CD4\(^{+}\) T cell response has been identified in 1994 in melanoma tyrosinase\(^{557}\). Since 1998, 27 new class II HLA-restricted epitopes of tumor antigens have been identified\(^{558,559}\).

**Fusion proteins:**

This has been reported in several malignancies particularly in some form of leukemia, the molecular mechanism of carcinogenesis involves translocation of chromosomes that results in fusion of distant genes. This has been shown to cause the synthesis of fusion proteins which characterize each type of disease (eg., bcr-ab 1 and pml-RAR in CML\(^{560}\) and APL\(^{561}\) respectively) and generate new epitopes that can be recognized by T-cells, either CD8\(^{+}\) or CD4\(^{+}\) in Class I or Class II HLA restriction, respectively.

**Brain Tumor Antigens**

Over the last decade, several ideas have emerged about the genetic alterations occurring in human cancer and how it leads to tumorigenesis.

Since the description of MAGE by vander Bruggen et al., (1999)\(^ {562}\), several brain tumor antigens have been characterized. MAGE family members have been shown to be expressed by some glioblastoma cell lines, that can be recognized by antigen specified T-cell, confirming the presence of MHC-peptide complexes at the surface of tumor cells\(^ {563}\). However, De Smet et al., (1994)\(^ {564}\) and Scarcella et al., (1999)\(^ {565}\) have shown no MAGE expression in culture tumors. This discrepancy between in vivo and in vitro data could be due to a different level of DNA
methylation induced by culture, since this regulates MAGE expression. Recently, some GAGE family members transcripts have been shown to be expressed in a high proportion of malignant astrocytoma, as well as medulloblastoma and ependymomas. However, Lethe et al., (1997) have pointed out that the recognition, and the lysis of tumor cells by specific T-cells needs a certain expression threshold of MHC peptide complexes, an important point that remains undefined in case of the GAGE.

In case of malignant gliomas, 'proteins' structurally altered during malignant transformation of glial cells play significant role. For example, the frequent p53 alterations observed early in the carcinogenesis of gliomas have been shown to provide new antigenic peptides that may trigger an immune response. Indeed, specific cytotoxic T-cell clones have been generated in vitro against mutated p53 protein, and in vivo immunization with a mutated p53 gene. Liberman et al., (1985) have reported a frequent molecular event in gliomas that the amplification and the mutation of the epidermal growth factor receptor (EGF-R) create new epitopes identifiable by mAb. However, it is not known whether such altered proteins can induce an immune response or serve as immune targets when expressed on glioma cells.

Recent studies have shown that in human malignant gliomas, phosphatase and tensin homolog (PTEN) and p16 tumor suppressor genes carry mutations. Alterations of these genes may contribute to gliomagenesis.

The PTEN gene, also known as MMAC 1 or TEP1, was identified in 1997 by Li, et al., as a tumor suppressor gene in various types of sporadic tumors, including malignant gliomas. This gene, deleted on chromosome 10, consists of 9 exons with exon 5 coding for the phosphatase core motif-terminal phosphatase domain. Steck et al., (1997) later isolated the same gene independently and designated the gene as MMAC 1 for 'mutated in multiple advanced cancers 1'. Li et al., (1997) identified the PTEN gene, TEP1 for TGF-β (transforming growth factor β) regulated and epithelial cell enriched phosphatase. Mutations were detected on exon 5 and 6 of the PTEN gene. Schmidt et al., (1999) have reported that mutations are distributed along the entire gene. In gliomas, PTEN mutations are preferentially found in GBM. The PTEN aberrations are detectable in a low fraction in anaplastic...
oligodendroglioma, and when present, indicate a poor prognosis. The PTEN mutations are either rare or present in grade I and II astrocytic, oligodendrogial and mixed gliomas, gliioneuronal tumors, as well as low grade and anaplastic tumors.

Another tumor suppressor gene frequently implicated in human gliomas is p16 (MTS1/CDKN2A/INK4A). Genetic evidence have accumulated that the p16 gene, which is located in chromosome 9p21, is involved in tumorigenesis, and plays a major role in the cell cycle at the G1-S check point. The p16 gene was first demonstrated by Kamb et al., in 1994. This gene encodes a 156 amino acid, 15.8 kD protein that blocks progression of cell cycle. Loss of this protein function may lead to cancer progression by allowing unregulated proliferation. Mutations in exon 1 and 2 of the p16 gene have been detected in malignant gliomas. Alterations of the p16 gene are known to occur in many primary tumors through different mutations including homozygous deletion, point mutation and hypermethylation of the p16 gene promoter. In astrocytic tumors p16 mutations have been reported as a rare mechanism, while Walker et al., (1995) have reported that deletion of p16 was more frequent in high grade gliomas.

Researchers have identified a gene that appears to play a key role in facilitating the growth of gliomas. Igor Garkavtsev et al., (2004) have shown that a candidate tumor suppressor gene—ING4, is an important regulator of brain tumor growth and angiogenesis. ING4 expression was found to be greatly reduced in glioma specimens compared with normal healthy brain. Moreover, as ING4 expression decreased, tumor grade increased. ING4's ability to inhibit angiogenesis and tumor growth appeared to involve an interaction with NF-kappa B.
GLIOMA

Gliomas are the most common primary neoplasms of the central nervous system, accounting for almost one third of nervous system tumors diagnosed each year. Glioblastoma multiforme and anaplastic astrocytoma, which together comprise the majority of malignant gliomas, are the most common primary brain tumors in adults aged 40 to 60 years of age. Multimodality treatment combined resection, radiotherapy and chemotherapy could not significantly improve survival in patients and have stimulated interest in experimental approaches to the treatment of these insidious neoplasms.

Gliomas develop and remain within the brain, rarely metastasizing beyond its borders. Yet while they are sequestered from the majority of the immune system as a result of immune privilege, they are still able to cause a broad depression in host immunocompetence. This control of peripheral immune function by a malignancy, which arises in an immunologically privileged site, offers a unique opportunity to explore how malignancies can impair immune function through the action of soluble factors.

GLIOMA IMMUNOLOGY

It is now evident that T lymphocytes play an important role in the antitumor response. The expression of antigens on the surface of tumor cells that can be recognized by the cellular elements of the immune system is the indispensable condition needed to generate a specific antitumor immune response. Several tumor-associated antigens shared by histogenetically related tumors have been identified such as tenascin, gp240, an altered epidermal growth factor receptor isoform (EGFR VIII), tyrosinase, tyrosinase-related proteins 1 and 2, gp1000, MAGE-1 and MAGE-3. Several of these antigens have been shown to be capable of generating a tumor-specific immune response in vitro and have led the way to using immunotherapy in the treatment of patients with malignant gliomas.

Generally, gliomas not only express a variety of tumor-associated antigens, but also have the ability to present these antigens to T cells.
shown to express low level of class I MHC, which can be increased both in vitro and in vivo after appropriate stimuli, such as IFN-γ exposure. Therefore, gliomas are capable of presenting tumor-specific antigens to cytotoxic T cells via the class I MHC pathway. Most human glioma cells also have been shown to express Fas/APO-1 (CD95) and Fas ligand, which can cause glioma cells to undergo Fas/Fas-ligand-mediated apoptosis, the major mechanism of T-cell mediated cytotoxicity, and also microglial cell killing.

To date, efforts to reliably manipulate the immune system to promote tumor regression in the brain have been universally disappointing. Factors contributing to the ability of glioma to escape the host immune system are its poor overall immunogenicity, its failure to express specific glioma antigens and the modulatory effects of the tumor on the immune system. Local lymphocyte infiltration into glioma has been well documented. However, the correlation between the extent of T lymphocyte infiltration and its effect on prognosis is controversial. Whereas, some studies have strongly supported a relationship between the extent of lymphocyte infiltration and improved survival, others have shown no significant benefit. Histologically, little sign of tumor rejection has been noted in the presence of lymphocyte infiltration and as a result, these tumor-infiltrating lymphocytes are thought to be functionally compromised. Indeed, a range of immunological defects, particularly effecting cell-mediated immunity, has been identified in patients with malignant gliomas. These patients have been shown to exhibit cutaneous anergy and an abnormal delayed hypersensitivity, a reduced number of circulating T-lymphocytes, a depressed lymphocyte proliferative response to mitogen, a decreased antibody response, and a deficient antibody-mediated and T-cell mediated cytotoxicity in vitro. The fact that this immunosuppression can be partially reversed by surgical removal of the tumor, strongly suggests that the presence of tumor itself is a major factor responsible for this immunosuppressed state.

Studies supporting glioma-induced immunosuppression have shown a downregulation of T lymphocyte activity from T lymphocytes harvested from both autologous brain tumor patients in the presence of glioma supernatants. It is now evident that the impaired lymphocyte responses in glioma patients are a result of
immunosuppressive factors produced by glioma cells in situ. Gliomas have been shown to synthesize and secrete multiple factors that are capable of inhibiting T-cell responsiveness. These include transforming growth factor-β (TGF-β2), Prostaglandin (PGE2), Interleukin-10 (IL-10) and Gangliosides (GANGS).

> **Role of TGF-β in immune suppression:**

Of the numerous immunosuppressive factors identified to date, the most well-characterized is transforming growth factor-beta 2 (TGF-β2), originally called glioblastoma cell derived T cell suppressor factor. TGF-β2 mRNA and its protein have been found to be greatly overexpressed in human glioblastomas and virtually absent from normal brain tissue. TGF-β2 has been shown to have potent immunosuppressive effects, including the inhibition of T and B cells proliferation, IL-2 receptor induction, cytokine production, natural killer cell activity, cytotoxic T lymphocyte development, and lymphokine-activated killer (LAK) cell generation. More important, it has been shown to directly inhibit the cytotoxic response of tumor-infiltrating lymphocytes. Furthermore, production of TGF-β2 by glioma cells may be a factor responsible for the low response seen in tumor-infiltrating lymphocytes isolated from brain tumors when exposed to stimulants such as lectin and Con A. TGF-β2 also downregulates the expression of the class II antigen HLA-DR (MHC), possibly another mechanism contributing to the tumor cells ability to escape immune surveillance. In addition, evidence shows that TGF-β2 is an important growth promoter of malignant glioma cells that exhibit TGF-Type I and II surface receptors. This phenomenon is believed to be caused by TGF-β2 induced promotion of angiogenesis and tumor-stroma formation, as well as an autocrine stimulatory effect on tumor growth.

> **Role of IL-10 in immune suppression:**

The role of IL-10 in the immune system is controversial. On the one hand, it has been suggested that IL-10 acts to inhibit the release of IFN-γ, IL-1α, IL-1β, IL-6, IL-8, GCSF, GM-CSF and TNF-α by lymphocytes and monocytes, partially
inhibits MHC-class II expression by monocytes, thereby hindering antigen stimulated proliferation and migration\textsuperscript{618,619}. On the other hand, IL-10 has been shown in animal models to enhance an effective and specific antitumor immune response\textsuperscript{620}. Further studies are necessary for better understanding the role of IL-10 in tumor-induced immunosuppression.

\textbf{Role of PGE\textsubscript{2} in the immunosuppression:}

PGE\textsubscript{2}, a product of arachadonic acid metabolism, has been shown to have profound modulatory effects on the immune system, including downregulation of the lymphokine activated killer cell activity and suppression of T cell proliferation\textsuperscript{621,622}. PGE\textsubscript{2} also downregulates the expression of the class II HLA-DR, possibly contributing to the ability of tumor cells to escape immune surveillance\textsuperscript{623}.

\textbf{Role of GANGS in the immunosuppression:}

Since GANGS are produced by gliomas and they modulate lymphocyte responsiveness, it is interesting to speculate that immunosuppression by GANGS might account for some of the dysfunctions observed in glioma patients. GANGS are sialic-acid containing glycosphingolipids\textsuperscript{624}. GANGS are components of human plasma with GM\textsubscript{3} and GD\textsubscript{3} being major constituents\textsuperscript{625}. They bind to both plasma proteins and lipoproteins\textsuperscript{626,627}. These are highly immunosuppressive moieties affecting both APC and T cell function\textsuperscript{628}. GANGS are shown to inhibit the expression of CD4 on human and murine T-cells as well as inhibit the generation of both cytotoxic T lymphocytes (CTL) and NK cell activity\textsuperscript{629}. Over the years, it has been shown that some tumors overproduce GANGS or produce GANGS with modified structure\textsuperscript{630,634}. Because tumor shedding of GANGS is known to occur, elevated levels of glioma-derived GANGS may accumulate peripherally in these patients.

\textbf{Role of other molecules in glioma immune escape mechanism:}

Glioma cells have been observed to form glycosaminoglycan (GAG) coat when cultured together with allogenic peripheral blood mononuclear cells in mixed
lymphocyte tumor cultures. These cells were found to be surrounded by clear 'pericircular halos' representing GAG coat. This contributes to this class of human solid tumors to evade cellular immune attack.

Recently CD-70, a tumor necrosis factor related cell surface ligand expressed on glioma cells also plays crucial role in immune escape mechanism. Its ligand CD27, expressed on T and B-lymphocytes, can induce apoptosis of the immune effector cells.

The immunologic consequences of primary malignant brain tumors clearly reveal a functional link among T cells, monocytes, and soluble mediators secreted by gliomas. Elucidation of the modulatory role of glioma derived suppressor factors which affect immune cell function is paramount to the understanding of the immunobiologic consequences of harboring a glioma. An understanding of these complex interactions will greatly aid in the development of strategies to treat these patients.

**MOLECULAR PATHOGENESIS OF GLIOMA**

One of the most important advances in neuro-oncology in the last decade has been the elucidation of the molecular anatomy of different glial tumors. Kraus and colleagues reported further details of these molecular differences (Table-3) by corroborating that p53 mutations, nuclear accumulation of p53 protein, PTEN and CDKN2 mutations, epidermal growth factor receptor (EGFR) amplification, and 10p and 10q loss are characteristic of patients with microscopically typical glioblastoma multiforme (GBM) and short (8-14 month) survivals. By contrast, in the 5% to 25% of patients who have GBM with oligodendroglial features, survival is often markedly improved. In Krau's series, the tumors in 3 of 13 such patients lacked the typical GBM molecular markers, and instead showed loss of heterozygosity (LOH) on chromosome 1p. These findings further support the evolving concept that primary glial tumors are derived from a common, multipotential precursor cell, and that the microscopic classification of tumors into GBM, anaplastic astrocytoma (AA), astrocytoma, anaplastic oligodendroglioma and oligodendroglioma is inadequate to
Several common genetic alterations at the chromosomal level have been observed including loss of chromosomes 17p, 13q, 9p, 19, 10, 22q and 18q and amplification of chromosomes 7 and 12q. These alterations led to changes in the expression of several genes during the genesis and progression of human gliomas; genes affected include p53, RB, IFN-γ, CDKN2, MMAC1, DCC, EGFR, PDGF, PDGFγ, MDM2, GLI, CDK4, and SAS. Analysis of recent studies suggests that altered expression of several other genes and proteins is associated with the genesis of human gliomas, those genes include MET, Myc, TGal, CD44, VEGF, hNr-CAM, NCAM-L1, p21Waf1/Cip1, trk A, MMRs, C4-2, and D2-2; and the proteins include cathepsins, tenascin, matrix metalloproteases, tissue inhibitors of metalloproteases, nitric oxide synthetase, integrins, IL-13 receptor, Connexin 43, uPARs, extracellular matrix proteins and heat-shock proteins. Taken together, these findings point to the accumulation of multiple genetic mutations coupled with extension changes in gene expression in the development of human glioma.

Table-3: *Genetic alterations in primary glial tumors* [Source: Michael Glantz 2003]
Although still incompletely understood, astrocytes undergo transformation with the loss of tumor suppressor genes critical for cell growth, differentiation and function. These genes are p53, the retinoblastoma (RB) gene, the INK4a (inhibitor of cyclin-dependent kinase 4) gene, and the PTEN gene. In low-grade astrocytomas p53 is inactivated by mutation or gene deletion. The progression into anaplastic astrocytoma (AA) and GBM (hence secondary GBM) is accompanied by RB and PTEN mutations with cell aneuploidy and overexpression of cyclin dependent kinase 4 (CDK4). Generally, secondary GBMs predominate in younger patients and represent 40% of all glioblastomas.

In primary GBM, the sequence of genetic changes is different with a final, identical phenotype. Primary GBM tend to show amplification of the epidermal growth factor receptor (EGFR), deletions in the INK4a gene with loss of p14 and p16 and diploid cells. The protein encoded by the RB gene regulates the cell cycle by inhibiting progression beyond the G1/S restriction point. Mitogenic signals activate a molecular cascade known as Ras mitogen activated protein kinase (Ras/MAPK). MAPK inhibits protein encoded by RB and activates the transcriptional factor E2F, and cell enters the S phase. The IN4Ka gene converges on the RB pathway by activating three cyclin kinase inhibitors; p1, p16 and p19. They inhibit a family of kinases known as cyclin-dependent kinases 2, 4 and 6, triggering cell-cycle progression by inhibiting RB encoded protein. Thus, the RB pathway plays a major role in the phenotype of AA and GBM. Several other factors like the EGFR and the vascular endothelial growth factor (VEGF) bind to specific tyrosine kinase receptors with downstream effects resulting in typical final tumor behavior or proliferation, invasion and angiogenesis.

The loss of heterozygosity in chromosome 10q causes inactivation of PTEN, a gene downstream of focal adhesion kinase (Fak), which controls cell migration and invasiveness. This effect is mediated by activating Akt, a serine/threonine kinase involved in cell proliferation and survival. Although primary mutations in Ras are less common or absent in glial tumors, there is over-expression or constitutive activation of other receptor tyrosine kinases as well as autocrine loop that in turn
activate Ras, spawning interest in the modulation of Ras signal transduction cascade as a therapeutic approach 640, 641.

EXISTING THERAPEUTIC MODULES OF BRAIN NEOPLASMS

Brain tumors often represent ‘difficult to treat’ neoplasms and are a major cause of death among children and adults between 40 to 60 years of age. Malignant brain tumors i.e. gliomas account for approximately one third of all diagnosed brain tumors 642. The poor prognosis associated with malignant primary brain tumors treated with conventional therapies such as a combination of surgery, radiotherapy and chemotherapy has led investigators to develop new innovative therapies. Standard treatments for malignant gliomas are often ineffective because of dose-limiting toxicities to the central nervous system and detrimental side effects that significantly alters’ a patients’ quality of life.

Surgery:

Brain tumors are generally not detected at an early stage, usually having 50gm in weight at the time of diagnosis. When diagnosed, surgical resection is seldom adequate unless the tumor is located entirely within a respectable lobe of the brain. It is clear that the location of the lesion tends to be the most important factor in determining the nature of symptoms and treatment module. Radical or total resection is the surgical procedure of choice for all forms of gliomas, because it affords the best prognosis in majority of patients 643, 644. Surgery is a primary treatment for accessible brain tumors, if the patient is in otherwise good health. Accessible tumors are those that can be surgically removed without causing severe neurological damage. Tumors located in gray matter or deep within the brain may be inaccessible. The goal of surgery is to remove all visible tumors. Many benign tumors are treated only by surgery. Most malignant tumors require additional treatment. The purposes of surgery
are: 1) to remove as much tumor as possible; Even partial removal of a tumor may provide for: relief of symptoms, improved quality of life, and fewer tumor cells for other treatments to control. 2) to establish an exact diagnosis; A sample of the tumor is removed (biopsy) for examination under a microscope in the laboratory so an exact diagnosis can be established. 3) to provide access for other treatments; During surgery, chemotherapy or radiation, implants may be inserted into the tumor bed. Hyperthermia (heat) treatments may be delivered. Preparation for investigational BCNT (boron neutron capture therapy) may be provided. Altered genes, another investigational treatment, may be placed at the site of the removed tumor. Tumor resection (when feasible) is also useful for reducing mass effect, which may be the major cause of symptoms and/or neurological deficit. Surgical resection of the tumor may also decrease the need for steroid therapy, allow a decrease of radiotherapeutic portal size, increase the effect of chemotherapy and limit sampling error that may occur in cases in which a biopsy sample alone is obtained. A large cranial opening is typically made to facilitate optimum exposure and the use of multiple trajectories, as well as aid in decompression of the brain.

Biopsy is a surgical procedure to remove a small sample of tumor for diagnosis. The sample is examined under a microscope by a pathologist who determines the exact classification of the tumor. A biopsy may be performed as part of the surgery to remove the tumor, or as a separate diagnostic procedure. For areas considered being "inoperable," the surgeon is often able to perform a biopsy through a small hole drilled into the skull, called a burr hole. Needle biopsy uses a narrow, hollow needle, which is passed through the burr hole. Tumor tissue is removed from the core of the needle. Nowadays, stereotaxic biopsy is the standard for obtaining a biopsy. Interestingly, Gianini et al., (2001) have reported that gross total resection or a biopsy procedure was associated with longer survival than subtotal resection. Image guidance using information from CT or MRI scans, provides precise information about a tumor's location and its position relative to the many structures in the brain. CT and MRI represent anatomical images for guidance of surgical procedures; other types of imaging can also be used such as angiography to define the vasculature of the tumor and its relationship with other vascular structures. Lately,
functional image is also being used such as PET, SPECT and functional MRI acquired under stereotactic conditions. Stereotactically guided equipment may be moved into the burr hole to remove a sample of the tumor. This is called a closed biopsy. Very important is to obtain more than one sampling, and the so called serial biopsies allows a well defined histological classification, as we have seen that gliomas often shows different grades and mixed histologies.

**Craniotomy and tumor resection:** The surgery to remove a brain tumor is called a craniotomy with tumor resection. The resection can be partial or complete. The neurosurgeon has a wide choice of tools to use in removing brain tumors. Commonly used tools are the surgical laser, ultrasonic aspirator, and operating microscope (microsurgery). Other tools include evoked potentials, ultrasound imaging, and stereotactic instrumentation. The choice of tools used depends on the type of tumor and its location. Special techniques allow maximum removal of tumor while protecting the brain from neurological damage or optimization of the surgical procedure. In general this special techniques are associated with minimal invasiveness. The most important techniques include the use of image guidance and physiological localization with brain mapping.

**Image guidance:** The purpose of image guidance is to provide precise information about a tumor's location and its position relative to the many structures in the brain. The classical precise form of image guidance is using stereotactic frames. As already mentioned, the type of imaging used includes both anatomical and functional images. The stereotactic technique can be used for serial biopsies, but also for craniotomy and tumor removal; the late, is called stereotactic craniotomy. To prepare for stereotactic surgery, a headframe is attached to the patient's skull. The rigid frame holds the patient's head in place during the pre-surgical scans and the surgery itself. The information from the CT and/or MRI scans, along with the coordinate information from the headframe, is entered into a computer system. The images produced, with their relational coordinates, are used to plan the surgery and guide the surgeon's tools during the procedure. A stereotactic craniotomy allows the neurosurgeon to center the craniotomy, locate the deep tumors and give invaluable information of depth of resection.
Over the past decade, advances in computer software have allowed the widespread use of stereotactic volumetric resection within the neurosurgical community. Functional magnetic resonance (MR) imaging, functional cortical mapping, awake surgery, image guided surgery (stereotactic craniotomy), and/or intraoperative MR imaging are modalities that may be used to increase the amount of tumor that may be safely resected. Kelly introduced the use of volumetric resection for gliomas approximately 25 years ago. Using a complete volume of images, and a computer, the technique was improved to be the so-called, computer assisted volumetric resection, where the information is used for trajectory and margin of tumors definition; on the later, a complete defined tumor volume is used to pre-plan the stereotactic approach. Additional instrumentation attached to stereotactic frames, such as stereotactic retractors has been used to keep the neurosurgeon oriented throughout the surgical procedure. Also heads-up display on the surgical microscope has been used to outline the tumor margins at different depths of the resection. Later developments include the so-called, interactive image surgery. This involves the use of volumetric images and also a localizer (or digitizer) technology that allows the demonstration of position of surgical instruments in a computer workstation with the preacquired images. This new concept brings to the operating room two components: a computer and the digitizer. One of the objectives of the digitizing technology was to eliminate the stereotactic frames, and for these reason it has been called "frameless" systems. Probably, the term is not appropriate as the stereotactic frame can be used as a reference for the digitizer and still surgery is performed as interactive image guidance. In general, the reference for interactive image guidance is markers, anatomical landmarks, etc. and corresponds to the so-called frameless techniques. Frameless navigational systems eliminate the necessity of a headframe. The surgeon touches a hand-held device against the brain during surgery. The device superimposes its location on a computer monitor showing a recent scan or three-dimensional image of the brain. This tool is used to orient the surgeon as to the exact location of the tumor as compared to a specific point on the exterior of the brain. The interactive image guided systems can be used to biopsy tumors, remove tumors, or to provide a navigational assistance during surgery. These
techniques are particularly useful in reaching a tumor located deep within the brain, or close to eloquent areas. Different type of localizers includes passive systems such as articulated arms, sonic, optic, electromagnetic systems and active systems.

One of the current limitations of stereotactic volumetric technology for resection of high-grade gliomas, however, is the phenomenon referred to as intraoperative 'brain shift'. Brain Shift during open neuro-surgical procedures occurs as the brain "relaxes", and this is related to different causes, including the use of mannitol, opening of the dura matter, cisterns, ventricles that caused cerebrospinal fluid diversion, and surgical debulking of large masses. This movement of the intracranial contents such that they do not match their perspective positions on pre-operative images has long been documented.

In one study, the brain surface shifted up to 2.4 cm during surgery; the amount and direction of shift depends on many factors, most importantly the volume of tissue resected. Benveniste et al., (2003) have shown that non-periventricular lesions were associated with a significantly greater rate of successful resection than periventricular tumors when the tumors is less than 30 ml in volume. Age was, however, not found to have any significant impact on extent of resection. So, frameless image-guided stereotaxy can be successfully used to assist in the resection of supratentorial malignant glioma (<30 ml), if precautions to minimize brain shift are respected. In cases involving large tumors and tumors located periventricularly, the use of intraoperative imaging to update in real time is desirable to increase resection accuracy. Unfortunately, despite all these development in resection technologies, the median survival time for patients after surgical intervention alone is six months, and only 5-7% patients survive for 2 years.

A complete resection of a malignant glioma is not possible either because of the location of the tumor adjacent to eloquent neural tissue or deep within the brain and because of the diffusely infiltrative nature of these tumors. Further, risk of debulking of areas controlling vital organs always remains, affecting patients' post-operative quality of life.
**Radiation Therapy:**

The role of radiotherapy remains quite controversial in the management of most gliomas. Although there is some evidence that low-grade ordinary astrocytomas may benefit from radiation therapy, radiotherapy has not been applied in a constituent fashion, and the dose-response relationship is not clear. Some investigators have used radiosurgery in an attempt to provide most focused delivery of radiation for low-grade gliomas, and in the future this may prove to have a role in combined management protocols.  

Many tumors are radiosensitive, their cells readily shrink and die when exposed to radiation. Radiation therapy is used: 1) following surgery when benign tumor is incompletely resected 2) following surgery for malignant tumor 3) instead of surgery for an inaccessible tumor or for a tumor that is particularly responsive to this form of treatment. Conventional external radiation therapy uses external beams of energy aimed at the tumor. The treatment usually begins one or more weeks after surgery and continues five days a week for about six weeks. This type of therapy is usually recommended for a large or infiltrating tumor. Varying dosage and schedules of radiation therapy are also used in special circumstances. Two variations of standard external radiation therapy are (a). **Hyperfractionated radiation** is the use of smaller-than-usual daily doses in order to deliver higher total doses of radiation. (b). **Conformal radiation** uses high-dose radiation beams "conformed" to match the tumor's shape. Computer-customized collimators, blocks, wedges, or the Peacock System selectively blocks the beams from a linear accelerator. Conformal radiation can also treat multiple tumors simultaneously. Different methods of delivering highly focal radiation are also in use. The rationale of all these techniques is to deliver a high dose of radiation to the tumor (much higher that conventional radiation therapy) at the same time sparing the normal surrounding brain tissue. For this highly accurate delivery of radiation, image guided stereotactic techniques have been used. The most common are: interstitial radiation, intracavitary radiation desmoplastic and radiosurgery. Other forms of radiation therapy, such as **Boron neutron capture therapy** (BNCT), are under investigation. The potentially interesting technique of Boron Neutron Capture Therapy is undergoing evaluation in the treatment of
malignant glioma. The process depends upon the addition of a low energy thermal neutron to boron-10, which then disintegrates in a ‘capture reaction’ as a cleanly localized nuclear reaction, effectively destroying all cells in 5-10 micron vicinity. Tumors can be encouraged to take up certain boron containing compounds but there are difficulties in getting sufficient amounts of boron into large number of cells for the technique to be clinically effective.664

**Interstitial radiation** (also called brachytherapy) uses radioactive seeds implanted directly into the tumor site. 125-iodine seeds in Teflon rods can be stereotactically implanted into small, well localized tumors, such as metastases and left in situ for a specified duration, before being removed.665 In general, two approaches have been used: permanent implants and temporary implants. With temporary implants the dose rate usually is 40 cGy/hr with a total minimal peripheral dose of 6000 cGy. Because they are aggressive, temporary implants have been used mostly in malignant tumors and located in cortical subcortical areas, being contraindicated in midline tumors. Clear improvement in survival has been shown with the use of temporary implants as a boost after external radiation in-patients with GBM and less clear on anaplastic astrocytoma; there is also an improvement in survival with its use at the time of recurrence. The permanent implants are prescribed at a 4-5 cGy at the first hour with a total minimal peripheral dose of 8000-10000 cGy delivered throughout the life of the implant (usually a year for 1-125, with a median-life close to 60 days). For these reasons, permanent implants have been used in malignant tumors but also in the management of low-grade tumors. Permanent implants can be used in midline tumors and in general, are better tolerated than temporary implants.666

**Intracavitary radiation** is radiation delivery during surgery to the tumor bed. **Stereotactic Radiosurgery** is a convergent delivery of radiation therapy. This form of treatment allows precisely focused, high dose beams to be delivered to a small (usually 4cm or less in diameter) brain or spinal cord tumor in a single treatment session. The tumor can be located in an area of the brain or spinal cord that may be considered inoperable. If the treatment is delivered in multiple sessions, it is called stereotactic fractionated radiotherapy. Using special computer planning, this
treatment minimizes the amount of radiation received by normal brain tissue. In addition to the uses of radiation therapy discussed above, stereotactic radiosurgery may be employed as a local "boost" following conventional radiation therapy, for a recurrent tumor when the patient has already received the maximum safe dose of conventional radiation therapy; as a substitute for surgery for a benign brain tumor (such as a pituitary, pineal region or acoustic tumor); or for a metastatic brain tumor. Prior to treatment, the patient is fitted with a headframe. CT and/or MRI scans are done with the headframe in place to obtain information necessary for treatment planning. Once the planning is completed, treatment may begin.

Three types of machines can be used for radiosurgery: 1) **Gamma Knife** contains 201 radioactive cobalt sources, which can all be computer-focused onto a precisely defined target area. The patient is placed on a couch and then a large helmet is attached to the head-frame. Holes in the helmet allow the beams to match the calculated shape of the tumor. The couch slides into a globe that contains radioactive cobalt. The most frequent use of the gamma knife has been for small, benign tumors, particularly acoustic neuromas, meningiomas and pituitary tumors. To treat a larger tumor, partial surgical removal may be required first. More recently, the gamma knife has been used to treat solitary metastases and small malignant tumors with well-define borders. However, the risk of radiation injury persists with this technique also. The risk increases as the tumor volume increases, and the localization of the tumor becomes more deep-seated. 2) **Heavy-charged particles using a Cyclotron**, which is an adapted nuclear reactor, that produces particle beams of protons, neutrons, or helium ions. These beams are used for a small, deep-seated tumor such as a pituitary tumor. The energy properties associated with particle beams make it effective for that type of tumor. The patient is positioned on a table with a fitted facemask in place. As the nuclear reactor smashes atoms, the released protons are directed toward the tumor through blocks, which have been computer, programmed to match the beams to the shape of the tumor. 3) **An Adapted linear accelerator** delivers a single, high-energy beam that is computer-shaped to the tumor. The patient is positioned on a sliding bed around which the linear accelerator circles. The linear accelerator directs arcs of radioactive photon beams at the tumor.
pattern of the arc is computer-matched to the tumor’s shape. This reduces the dose delivered to surrounding normal tissue. Dosage can be adjusted by degree during the arc treatments. Moving the treatment bed under computer control allows flexible positioning. Typically, a single dose fraction is used each day until a total dose of 60 cGy is delivered. However, whole brain radiation is not recommended for most patients with malignant brain tumors because of the potential of severe side effects like dementia. For most malignant gliomas, normal tissue tolerance of brain white matter has been found to limit the effective radiation dose. Interstitial procedure is not always advantageous, whereas permanent brachytherapy is recommended, where the symptomatic necrosis appears to be low.

Although, radiotherapy is believed to prolong patients’ postoperative survival, after 12-18 months, there is little difference in the survival rate of irradiated and non-irradiated groups. Detrimental effects in long-term survivors have included personality change, memory loss, dementia, hypopituitarism, gait abnormality, urinary discomfort, coordination and/or balance difficulty, and radiation necrosis with mass effect. Olson et al., (2000) have future reported that in a study including 77 oligodendrogloma patients, 21% developed radiation-induced cognitive change and 15% developed necrosis. Reducing the total radiation dose, daily fraction sizes (hypofractionation), and/or size of the fields, or the use of three-dimensional conformal therapy, any help reduce the incidence of complications.

Though radiotherapy helps but has serious side effects, the critical issue is whether it should be administered postoperatively, or reserved for later, either after chemotherapy or at the time of tumor progression. Henderson (2001) and Leighton (1997), have indicated that delayed radiotherapy is as effective as postoperative therapy in treating patients with low-grade oligodendrogliomas, provided that minimal residual tumor is present. Moreover, Veninga et al., (2001) have reported that repeated radiotherapy can be considered as a treatment option in good-condition patients with recurrent gliomas, including oligodendrogliomas.

Finally, it is evident that the decision to perform radiotherapy is made on a case-by-case basis, considering factors such as the extent of resection, tumor location, and the patient’s age and occupation.
Chemotherapy:

Chemotherapy uses special drugs to kill tumor cells. The drugs may be given by mouth, injected into the bloodstream through a vein or artery, or placed directly into the cerebrospinal fluid. Currently, many clinicians are administering chemotherapy before, during or after radiotherapy and then again at the time of tumor progression. During surgery, drug-saturated wafers may be placed at the tumor site. Chemotherapy may be used before, during or after surgery and/or radiation therapy. Recurrent tumors are treated with chemotherapy as well. In very young children, chemotherapy is used to delay radiation therapy to avoid potential injury to their developing brains. Many drugs have anti-brain tumor activity. Nitrosourea agents seem to be the most logical choices at the present time in the treatment of especially low-grade gliomas. These drugs are used as single agents or in combination with other drugs, such as procarbazine, lomustine and vincristine (PCV). Multiple chemotherapy with cisplatin, Ara-C and Etoposide has been found to exhibit limited survival benefit especially in case of recurrent pediatric brain tumors. But it is difficult to predict which tumors will respond to which chemicals and to what degree. Temozolomide have also been tested in patients with anaplastic astrocytoma and glioblastoma multiforme. Boutros (2000) & vander Bent (2000), reported the use of conventional-dose-temozolomide in 30 patients with recurrent oligodendrogliomas and oligoastrocytoma. 27 of these patients had previously received PCV chemotherapy, but only 9 responded, suggesting that these patients constituted a prognostically unfavorable group. Results further indicated that prior response to PCV did not predict response to temozolomide. Again toxicity was mild and predictable. For this reason, treatment often consists of a combination of drugs. Tumor cells are vulnerable to chemotherapy because cells that are actively dividing absorb the drugs used. But some normal cells may also be affected which is what causes side effects. The cells that produce blood, hair, skin and that line the digestive system also are actively growing. Chemotherapy is usually given intermittently over a scheduled period of time. This schedule allows normal cells to recover between treatments. Many forms of chemotherapy are under investigation.
Recent studies have identified presence of chemoresistant genes in the brain tumor cells, which hampers the role of chemotherapy in successful control or reduction of tumor burden (Table-4)\(^687\). Moreover, several mechanisms of drug resistance have been found to limit the cytotoxic effects of these chemotherapeutic agents. The first mechanism is the repair of alkylator-based DNA crosslinks by O\(^6\)-alkylguanine DNA-alkyl-transferase (AGT), a protein present in tumor cells that mediates the repair process\(^688\), and the second mechanism involves the failure of the cell to conduct DNA mismatch repair. The roles of these mechanisms in the sensitivity of malignant gliomas to alkylating agents have been demonstrated in human brain tumor xenograft models\(^689,690\). The administration of chemotherapeutic agents is also hampered by the presence of blood-brain barrier, which prevents the passage of molecules that are larger than 180kD into the brain\(^691,692\). Intra-arterial drug delivery with or without the use of hyperosmotic agents, drug delivery by stereotaxic procedure directly into tumors, are some of the area where research is going on in case of CNS tumors.

<table>
<thead>
<tr>
<th>Chemotherapeutic agent</th>
<th>Chemoresistance producing genes</th>
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<tr>
<td>Molecule CENU</td>
<td>Mgmt gene</td>
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<tr>
<td>ACNU</td>
<td>Mgmt gene</td>
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<tr>
<td>Vincristine</td>
<td>Mdr I</td>
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<tr>
<td>Etoposide</td>
<td>DNA topoisomerase II gene</td>
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<tr>
<td>Cis-diaminedichloroplatinum (CDDP)-II</td>
<td>Metallothionine IIA gene, GST-[\pi] gene</td>
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<tr>
<td>Multiple Drug Resistance (MDR)</td>
<td>MRP genes</td>
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Table-4: Different chemotherapeutic agents and the respective chemoresistant producing genes

The goal in delivering targeted toxins to brain tumors is to achieve diffuse homogenous distribution of the agent throughout the tumor and into the adjacent area of tumor cell-infiltrated brain parenchyma\(^693,694\). In this process of targeted toxin
delivery, using a high-flow microfusion technique, volumes of up to 180 ml were infused by Convection-Enhanced Delivery (CED) through catheters placed directly into brain tumors. Neuronal toxicity manifested as seizure activity and hemiparesis resulted from peritumoral oedema that followed the completion of the infusion. Peritumoral toxicity was believed to be more related to the concentration of the infused immunotoxin than to the infusion volume. In approximately half of the patients treated with CED, either a stable disease course or a partial response or a complete response was found in some clinical trials.

Targeted toxin therapy by CED appears to have therapeutic efficacy in patients with malignant gliomas. Direct intratumoral delivery of these chemotherapeutic agents appears to be the best route for their administration. The optimal dosing regimens that include the flow rate, infusion volume and concentration of the infused agent have not been firmly established and will vary based on which targeted toxin is chosen for treatment.

As with radiotherapy, chemotherapy is associated with significant adverse effects. Side effects of PCV (procarbazine, lomustine and vincristine) chemotherapy have been reported to include, nausea, vomiting, anorexia, fatigue, rash, numbness or paresthesias, weakness, abdominal pain, constipation, neuropathy, hepatotoxicity, encephalopathy, seizures, intracranial haemorrhage, pneumonia and other infections caused by neutropenia and thrombocytopenia. Olson et al., (2000) have reported that in a study, 46% of the patients treated with PCV chemotherapy developed myelosuppression.

Given the potential benefit of chemotherapy, until new data become available, PCV chemotherapy continues to be an option especially for the patients with low-grade oligodendroglioma. Therefore, chemotherapy lacks efficacy in most histological types of primary human brain tumors and has, for most types, failed to improve outcome for patients. The unsatisfactory results with chemotherapeutic intervention in these cancers have been chiefly attributed to tumor-cell resistance. Elucidation of the cellular mechanisms involved in resistance regulation is needed for future progress in efficient approaches to selective modulation of drug resistance in these lesions.
The dismal prognosis for patients harboring malignant intracranial tumors despite multimodality treatment combined resection, radiotherapy and chemotherapy has prompted an intensive search for effective treatment alternatives such as immunotherapy.

**IMMUNOTHERAPY:**

Over the years, numerous attempts have been made to establish an effective form of immunotherapy for the treatment of brain tumors. Both passive and active immunotherapeutic approaches have been attempted, alone or in combination, in patients with gliomas. Historically, early trials focused on using whole tumor cells, antibody, and/or nonspecific adjuvant such as Bacilli Calmette-Guerin (BCG) and Corynabacterium parvum (now known as Propinobacterium acnes, P.acnes), and resulted in little success. Later, the discovery of T-lymphocyte growth factor (IL-2), lymphokine activated killer (LAK) cells, tumor infiltrating lymphocytes, and tumor-sensitized cytotoxic T cells generated a new wave of enthusiasm for adoptive immunotherapy. However, to date, no trial using these new innovations has unequivocally demonstrated an improved overall survival for patients with malignant gliomas. The latest approach of immunotherapy, termed ‘BRM therapy’ has revolutionized the brain tumor immunotherapy. Following ablative therapy, application of certain biological compounds, i.e. Biological Response Modifiers (BRMs), either of exogenous or endogenous origin has been administered with more or less success at the initial stage. The principal rationale of this fourth modality of therapy (i.e. BRM therapy) lies in the belief that BRMs derived from ‘natural sources’ should provide precisely the type of measured, biological control to treat successfully a disease process and that these heterogeneous group of substances should be less harmful to patients than chemically synthesized chemotherapeutic agents.

The BRM modulatory approaches could be grouped under four generally accepted modalities:

1. Restorative immunotherapy
2. Adoptive immunotherapy
3. Restorative Immunotherapy

Restorative Immunotherapy encompasses the largest number of approaches involving BRM agents and quite frequently employs microbiological organisms or their active components in an attempt to arouse or heighten activity of immune-related cells nonspecifically. The rationale lies in the belief that non-specific systemic immunological activation will result in immune recognition of tumor antigens as foreign with subsequent focused antitumor responses. Some investigators have shown that the interaction of immune-related cell in patients with brain tumors may be weak due to an inability to secrete sufficient lymphokines. Further, the mitogenic activation of peripheral blood T lymphocytes is also impaired. This has been attributed to a paucity of high-affinity cell surface receptors for interleukin-2 (IL-2). In view of this, natural biological agents such as various cytokines (interferons, interleukins, and thymosins) have also been used in a broad attempt to startle immune-related cells into action or to supplement low or negligible levels of these soluble mediators.

Restorative immunotherapy with microbial or synthetic agents alone in patients with malignant brain tumors has been disappointing, but, since these agents are relatively safe and well tolerated, their use as an adjunct with other therapeutic modules has been suggested. Among microbial agents used for restorative immunotherapy in malignant brain tumor patients are BCG, BCG-cell walls (BCG-CW), Corynebacterium parvum (P. acnes), OK-432 (Picibanil) and mumps virus. Treatment of patients with BCG organisms and BCG-CW together with other therapy yielded no indication of efficacy. Selker et al., (1978) treated six patients with anaplastic gliomas with C.parvum (5mg/m²) given intravenously, but no neurological improvements were reported. Ishizawa (1981) and Shibata (1987) treated glioma patients with Picibanil in addition to radiation therapy and ACNU chemotherapy, but no efficacy was observed. Mumps virus vaccination in patients with low-grade gliomas showed some form of clinical improvements.
Local administration of cytokines (Interferons, Interleukins) has been shown to exert potent anti-tumor activity against a wide range of malignant brain tumors. Interferons constitute at least three separate families of closely related glycoproteins with anti-viral, immunoregulatory and anti proliferative properties.

Human Interferon-alpha (IFN-α) has been shown to affect the growth of human glioma cells in vitro. Salford et al., (1981) reported the use of IFN-α in brain tumor patients was without toxicity. Mahaley et al., (1985) reported CT scan evidence of response in seven of 17 patients with recurrent gliomas.

Interferon-beta (IFN-β) was the first of the interferons to be extensively studied clinically in patients with gliomas. IFN-β has been shown to inhibit replication of human glioma cells in vitro and in nude mice. Clinical trials have suggested that a combined route of intravenous and intratumoral administration of IFN-β was more effective than intratumoral alone. Yoshida et al., (1986) have reported treating nine cases of brain tumors with radiotherapy, ACNU and IFN-β with four complete responses and one partial response. Evidence of tumor regression was reported in some of the patients after the use of a recombinant form of IFN-β that has been genetically modified to make the molecule more stable.

Interferon-gamma (IFN-γ) has been shown to increase the HLA-DR antigens on glioma cell surfaces and to activate lymphocytes to produce specific toxicity in the presence of IL-2. Reports of using intraventricular IFN-γ after postoperative radiotherapy in three patients with gliomas are also there. One patient showed a gradual reduction in tumor size. However, it was not clear whether the response was due to IFN-γ alone or due to proximity to radiotherapy. In glioma patients, where the immune system is severely suppressed, IFN-γ might be an effective therapeutic agent as it can stimulate various components of the immune system. In addition, recombinant IFN-γ has been shown to stimulate the production of tumor necrosis factor (TNF) by human peripheral blood monocytes in vitro. IFN-γ can also help greater lymphocyte infiltration in the brain by activating endothelial adhesion molecules. However, IFN-γ may be more effective when administered with other cytokines or chemotherapeutic agents. In spite of its immunomodulatory effects, the
associated toxicities and adverse side effects posed limitation for the use of IFN-γ in therapeutic purpose.

Interleukins represent group of soluble immune messengers that specifically stimulate immune related cells to amplify a specific immune response through proliferation and/or differentiation into immune effector cells.

Interleukin-1 (IL-1), an inflammatory cytokine has been regarded as a true immunostimulant that can regulate functional responses of immune-related cells (T and B-lymphocytes, macrophages) non-specifically. In addition, IL-1 induces other lymphokines and cytokines activities. Profound immunomodulatory activity coupled with its antitumor cytotoxic activity makes IL-1 an excellent anti cancer BRM. Its principal mode of action appears to be its capacity to induce synthesis of prostaglandins, especially PGE, which in turn suppress the immune system, which mediate a variety of physiological changes. However, IL-1 could yield life-threatening toxicities, so it is not likely to be used to treat patients in vivo. Rather, it could function as in vitro modulator of various immune-related cell responses prior to auto-adoptative therapy.

Interleukin-2 (IL-2) has been the most useful interleukin for BRM-related therapeutic approaches. IL-2 is required for the growth of C8+ T lymphocytes, and it stimulates the cell to engage in cytotoxic activity. It enhances the activity of NK/LAK cells, which process antitumor activity, whereas the CD8+ cells provide antigen-specific antitumor functions. The use of IL-2 in vivo alone and in combination with LAK cells generated in vitro was also demonstrated. The animals treated with IL-2 have shown to exert a strong immunological cytotoxic anti-tumor response, leading to significant prolongation of survival time. However, adverse effects of systemic IL-2 vaccinations in glioma patients were observed, the most notably being the 'capillary' leak syndrome. IL-2 causes an increase in vascular permeability that leads to accumulations of fluid in body compartments and possibly to intracerebral oedema. Because the administration of IL-2 does not result in neutropenia or immuno-suppression, patients are not susceptible to the opportunistic infections frequently observed following administration of chemotherapeutic agents, but adverse side effects like fever, chills, tachycardia, diarrhoea, fluid retention, are
observed in patients with brain tumors. Therefore, in most cases IL-2 is given concomitantly with LAK cells and adjunctively thereafter to prolong or maintain LAK cell activity in vivo. CNS toxicity that commonly occurs with this form of treatment also limits the amount of IL-2 that can be administered.

Interleukin-4 (IL-4) or B cell growth factor specifically stimulates antibody production by the B-lymphocytes and may become an essential ingredient in cytokine-based immunotherapy of glioma patients. Significant inhibition of tumor growth in nude mice was observed when the tumor and IL-4 secreting cells were injected intracerebrally. It has also been shown to synergize with IL-12 and GM-CSF in the induction of anti-glioma antitumor responses.

Interleukin-12 (IL-12), activates the antigen-presenting cells for anti-tumor rejection. Local delivery of IL-12 into rat brain by genetically engineered cells has been shown to prolong survival time in animals with malignant gliomas. Although with IL-12 effective anti-tumor activity is observed, combination of vaccine therapy with IL-2 produces better results. Thus the weakly antigenic neoplastic cells may become immunogenic stimulating anti-tumor immune response as a result of the presence of these cytokines, but the associated toxicity limits the use of IL-12 cytokine therapy.

Several other cytokines like IL-15, IL-13 and IL-18 has also been characterized to exhibit anti-tumor activity in various types of gliomas.

It has been suggested that IL-13 and analysis of IL-13 receptors may have clinical applications in glial tumors. IL-15, in contrast, activates tumors specific gamma delta T cells, indicating its role as an adjuvant in immunotherapy of brain tumor patients. IL-18, a potent IFN-γ inducing cytokine has been shown to effectively regress tumor load in naïve mice after a single dose of recombinant IL-18.

However, the severe adverse effects and toxicities present serious limitation in the use of interferons and interleukins in restorative immunotherapy of brain tumor patients.
2. **Adoptive Immunotherapy**

Adoptive immunotherapy is defined as the administration of sensitized immune cells, either autologous or allogeneic, in an attempt to transfer specific immunity to a patient with absent or suppressed immunological activity. It can take two distinct routes with regard to the mechanisms used to sensitize the effector cells, namely specific adoptive immunotherapy and non-specific lymphokine activated killer (LAK) cell production.

Specific adoptive immunotherapy involves the administration of authochthonous lymphocytes that have been specifically stimulated *in vitro* with authochthonous tumor cells to generate an expanded population with a highly restricted cytotoxic activity. Specific adoptive immunotherapy has been readily demonstrated in animal models with a highly restricted cytotoxic activity. Specific adoptive immunotherapy has been readily demonstrated in animal models. A T-lymphocyte cell line that was specifically cytotoxic towards murine malignant glioma cell line, 203-glioma and restimulated with IL-2, effectively neutralized tumor growth. In another study, Miyatake et al., (1986) stimulated peripheral blood lymphocytes from glioma patient and induced two human T-lymphocyte cell lines that express glioma-specific cytotoxic activity. The PBL were co-cultured with the patients own tumor cells and IL-2. These two cytotoxic T cell lines with minimal reactivity towards non-glioma tumor cells and mitogen-activated autologous lymphoblasts killed both autologous and allogeneic glioma cells. These studies provide evidence that it is possible to generate specific antiglioma cytotoxic T lymphocytes *in vitro* and could be expanded for antiglioma therapy. In a recent study, Sloan et al., (2000) have reported that adoptive immunotherapy in which autologous whole-tumor cell vaccine is combined with GM-CSF (Granulocyte-macrophage colony stimulating factor) followed by adoptive transfer of CD3 and IL-2 activated lymphocytes is associated with evidence of tumor specific immune response and improved survival in patients with recurrent malignant glioma. The presence of anti-CD3 activates antigen-primed T lymphocytes and the effect on naïve T cells is minimal. Recent evidences further suggest that peripheral blood
lymphocytes are more effective in homing to tumor in situ than lymphocytes from vaccine-draining lymph nodes.

LAK cell activity, in contrast, is a composite effect of stimulating all mononuclear leukocytes non-specifically i.e., no attempts are made to stimulate the effector cells with any specific (glioma) antigens. LAK cells are peripheral blood leukocytes that have been activated in vitro with mitogens and/or IL-2. These cells are non-specifically cytotoxic to tumor cells but with a broader range of specificity than the natural killer lymphocyte that occur naturally in the peripheral blood. Jacobs et al., (1986a, 1986b) has reported somewhat beneficial results of preclinical studies with the 9L-glioma cell line in rats. Preliminary data concerning a clinical phase I trial in 39 brain tumor patients (18 with GBM) treated intracranially with LAK cells was also reported. With 30 patients still alive at the time of the report, they were able to document the minimal toxicity associated with this treatment modality. The use of tumor-infiltrating lymphocytes (TIL) in LAK generation in humans is another possible vehicle for immunotherapy of glioma. The rationale is that immune-related cells infiltrating the tumor bed are more likely to represent specifically activated effector cells. However, the poor proliferative responsiveness of glioma-derived TIL limits its use in LAK cell adoptive immunotherapy. Moreover, TIL and LAK cells are associated with low response rate possibly due to poor migration to the tumor site, as well as a significant incidence of toxicity. Production of LAK cells is also labour-intensive with high potential for microbial contamination of the cultured cells.

More recently, several groups have used dendritic cells pulsed with either tumor homogenate or tumor RNA to treat intracranial gliomas successfully in animals. To date, Dendritic cells (DC) are recognized to be the most potent antigen presenting cells (APCs) responsible for initiating an immune response. Dendritic cells are bone marrow derived cells similar to monocyte/macrophage and can be obtained from culturing peripheral blood mononuclear cells. Upon exposure to tumor cells, isolated tumor antigen, or even tumor mRNA, dendritic cells are capable of presenting endogenous and exogenous antigens to naïve T cells in an HLA-restricted manner. In addition to high level of expression of both MHC
class II molecules and I, dendritic cells possess the ability to secrete immune-stimulatory cytokines and express several adhesion and costimulatory surface molecules. All these leads to an efficient priming of the CD4+ T-helper cells and the generation of potent antitumor CTLs, which result in a specific protective and therapeutic antitumor immune response. Siesjo et al., (1996) showed that immunization with tumor cells mixed with syngenic spleen derived dendritic cells resulted in a significantly prolonged mean survival time for rats harboring intracranial gliomas. Later Liau et al., (1999) reported prolonged survival for rats with 9L gliomas after vaccination with dendritic cells pulsed ex vivo with acid-eluted protein from 9L glioma cells. Vaccination with antigen-loaded dendritic cells resulted in increased CD8+ T-cell infiltration into the tumor and increased 9L-specific CTLs. Similar results have also been reported when vaccinating mice with dendritic cells pulsed with Semliki Forest virus-mediated glioma complementary DNA, and dendritic cells fused to glioma cells. Intratumoral injection of dendritic cells (DC) and irradiated glioma cells (IR-GL) in a mouse brain tumor model elicited systemic immunity against autologous glioma cells and prolonged the survival of the tumor-bearing animal. Efficacy was reduced when studies were performed in mice depleted of CD8+ T-cells.

A number of clinical trials in patients with glioma were also done with DC vaccinations. Liau et al., (2000) published a case report on a patient with glioblastoma who was immunized with autologous DC pulsed with allogeneic MHC-class I matched tumor peptides. The tumor progressed 2 months after DC vaccination but on histologic analysis showed increased CD3+ T-cell infiltration with no signs of EAE. Later, the first phase I clinical trial using a DC-based vaccine for glioma patients was reported by Yu et al., (2001), where patients received autologous peripheral blood DC, which were prepared with IL-4 and GM-CSF, and pulsed with peptides eluted from the surface of autologous glioma cells. Patients showed prolonged survival time, possibly related to the production of an enhanced systemic cytotoxicity response and an increased intratumoral cytotoxic and memory T-cell infiltration. Very recently Kikuchi et al., (2001), reported a phase I clinical trial in which glioma patients were vaccinated with a novel fusion product of
autologous DC and glioma cells. Vaccination with the fusion cells increased CD16+ and CD56+ cells in peripheral blood lymphocytes and IFN-γ production in peripheral blood mononuclear cells.

Immunotherapy with DC proved to be safe, but in most of the studies reported, an array of tumor-associated antigens rather than single peptides were used because of the inability to identify a specific universal glioma antigen. Further research on the use of DC therapy as an adjuvant to current therapy for the treatment of patients with malignant glioma is warranted.

**GENE TECHNIQUE-BASED IMMUNOTHERAPY**

Advances in recombinant gene technology allowed the use of gene therapy for the treatment of patients with malignant tumors by providing a new method for the delivery of cytokines. Transduction of various cytokine-specifying genes into malignant cells as well as cytokine secretion by the cells themselves has also resulted in an augmentation of their immunogenic properties. Genetic constructs may be modified by viral vectors or plasmid DNA so that to express a variety of genes in vitro and in vivo that encode tumor antigens, cytokines, or accessory molecules. Genetic modification of tumor cells can increase their immunogenicity and potentially enhanced the systemic immune response generated against an intracranial tumor. Numerous cytokines such as IFN-γ, GM-CSF, or IL-12 have been tested.

Gene-based glioma immunotherapy is not limited to the expression of new genes. Vaccination with genetically engineered glioma cells expressing anti-sense molecules that block specific gene expression, such as glioma derived immunosuppressive factor TGF-β2 and insulin-like growth factor (IGF)-1, have also been shown to suppress intracranial tumor growth.

In addition to the subcutaneous vaccination of genetically engineered tumor cells, the effect of local cytokine production on intracranial glioma growth has also been examined. The direct intracranial implantation of tumor cells genetically engineered to secrete specific cytokines IL-2, IL-4, GM-CSF, TNF-α and IFN-γ have been shown to demonstrate survival advantage in animal model, while this approach has yet to be used clinically. Further, genetically engineered adenovirus and
herpes simplex virus expressing various cytokines have been tested, and again a
definite survival advantage was obtained when used in experimental brain tumor
models. Andréansky et al., (1998), have reported that a herpes simplex virus
modified for the expression of IL-4 resulted in a significant prolongation of survival
in mice harboring intracranial glioma. Furthermore, an inflammatory reaction was
observed in the brains of the treated animals, which was composed primarily of CD8+
and CD4+ T cells, suggesting a specific immunocytotoxic antitumor response. The
realization that several genes used by viruses in their lytic life cycle interact and/or
complement the function of genes employed by cells in cellular events suggests the
development of treatment strategies wherein viral mutants could be employed. Such
virus (designated as oncolytic viruses) can selectively grow in tumor cells, produce
viral progeny in those cells, lyse them and release their progeny that can then infect
additional cells in the tumor mass. In another study, Glick et al., (1995) demonstrated that mice implanted subcutaneously or intracranially with autologous
glioma cells mixed with allogeneic fibroblasts, genetically engineered to secrete IL-2
or IL-2/IFN-γ, developed systemic antiglioma cytotoxic immune response resulting
in prolonged survival. This approach avoids the need to transduce glioma cells from
individual patients and allows for gene-based immunotherapy to be administered
immediately after surgical intervention.

Despite these exciting preclinical results few clinical trials have been
reported. Sobol et al., (1995) reported on a single case of a patient with
glioblastoma multiforme treated with repeated immunizations using autologous tumor
cells and genetically modified fibroblasts using retroviral gene transfer to secrete IL-
2. An anti-tumor immune response, mediated in part by CD8+ cytotoxic T cells, was
demonstrated in the patient’s peripheral blood mononuclear cells and the patient
survived for 10 months after the initiation of immunization.

Despite its promise, gene therapy presents several potential barriers to clinical
application. The main barrier is the need to modify individual glioma cells taken from
each patient prior to treatment. The second concern is the ability, in vivo, of
effectively transfecting tumor cells with the viral gene product. This is of particular
concern to tumor cells along the edge of a glioma, which has infiltrated well into the
‘normal’ brain. In addition, re-implanting into a patient genetically engineered tumor cells with their potential to grow being unknown is a concern.

3. Passive Immunotherapy:

Passive immunotherapy is the humoral immune response equivalent of adoptive immunotherapy and is based on administration of serum or specific antitumor antibodies rather than cellular immune components. Major hurdles to be overcome in passive antiglioma antibody theory are those of producing “functionally or operationally specific” antibodies to glioma antigens and devising rational methods to deliver these antibodies within the tumor bed in useful quantities. While there have been no confirmed reports of a truly glioma-specific antigen, much progress has been made in defining, with both exhaustively adsorbed polyclonal antisera and carefully characterized monoclonal antibodies.

Specific antibodies have been used to vector a wide range of chemical and biological antitumor agents to malignant cell surfaces. Among these agents are radioisotopes, polyhedral boron for neutron-capture therapy, photo-reactive substances, bacterial or plant toxins and interferons. Early attempts with polyclonal antisera revealed specific tumor localization but these encouraging studies were frustrating and disappointing due to the lack of sufficient quantities of absorbed antibodies for therapeutic evaluation. The first study of radiolabeled antibody localization in 11 patients with malignant gliomas was conducted by Day, Mahaley and coworkers. In these studies, individual rabbit antisera were prepared, purified and radiolabeled from tumor tissue removed at original surgery. However, this was a limited trial due to several practical reason, like amount of effort needed to prepare exhaustively adsorbed specific rabbit antisera to each patient’s tumor, amount of antibody obtained and relatively low level of localization observed.

Monoclonal antibody (mAb) technology, which can provide unlimited quantities of highly specified antibodies, has fostered a renewed interest in this potentially important immunotherapy arena. Stavrou et al., (1986), have produced mouse mAb to rat MNU-induced glioma cells and determined that one of these,
14AC1, was capable of inhibiting growth of rat glioma cells in vitro and drastically reduced the number of lung metastases of pretreated and intravenously injected glioma cells. However, termination of mAb treatment led to increased tumor growth. Saya et al., (1985) have produced a syngeneic rat IgM tumor specific mAb (MoAb) (FR77) by immunizing Fischer 344 rats with syngeneic 9L/R, glioma cells. ADCC studies demonstrated weak but definite killing. Interestingly, the antibody also crosses species boundaries in that it binds selectively to some human malignant gliomas. Lee et al., (1986a, 1986b) characterized 4 syngeneic rat mAbs raised to a cloned avian sarcoma virus-induced glioma cell line of F344 rat origin, the mAbs appeared to recognize the same epitope on some, but not all, rat and mouse neurogenic tumors. The failure of the antibodies to identify the antigen on all clones of positive cell lines provides yet another example of the phenotyped heterogeneity among gliomas. de Vellis, Peng and coworkers have described production of mAb 2176 by immunizing mice with the C6 rat glioma cell line. A major obstacle that remains to multiple therapeutic administrations of murine mAbs is the avoidance of circumvention of the human antimouse antibody (HAMA) response in patients. The use of chimeric antibodies substantially reduced the HAMA responses but did not eliminate them.

Sikora et al., (1983), have produced a panel of 8 antiglioma human MoAbs in 23 fusion attempts using intratumoral lymphocytes. One of these antibodies has been used for specific localization studies in 12 patients with gliomas.

**Anticancer antibodies:**

Kohler and Milstein (1975) launched the modern era of targeted therapy for cancer in 1975 with the discovery of monoclonal antibodies. Therapeutic antibodies have become a major strategy in clinical oncology owing to their ability to bind specifically to primary metastatic cancer cells with high affinity and create antitumor effects by complement-mediated cytotoxicity (naked antibodies); or by the focused delivery of radiation or cellular toxins (conjugated antibodies). Finally, fully human antibodies have been developed using murine sources and transgenic techniques. As of late 2002, there were 6 anticancer therapeutic antibodies approved...
for sale in the United States. Most of them are effective against breast cancer, leukemia, lymphoma etc., while GliomAb-H and TriGem, having investigational indications against brain tumor are in the early state of clinical development \textsuperscript{794}.

4. \textit{Active Immunotherapy:}

Active immunotherapy requires active immunization with tumor-related antigens in an effort to increase the patient's ability to generate an anti-tumor response specific for the patient’s autochthonous neoplasm. This may involve autochthonous or allogeneic tumor-associated or tumor-specific antigens and quite frequently requires microbiological BRMs incorporated as strong adjuvants in order to elicit immunological responsiveness.

Bloom et al., (1960)\textsuperscript{795} conducted the first therapeutic attempt by subcutaneous implantation of autologous tumor cells in a patient with malignant astrocytoma. The attempt was unsuccessful as evident by the growth of viable tumor cells at the site of implantation. Later, other investigators\textsuperscript{796, 797} conducted studies on malignant glioma patients, but no difference in survival was observed. Trovillas & Lapras (1970)\textsuperscript{798} randomized 65 malignant glioma patients into 4 treatment groups; 17 patients received no postoperative therapy, 20 patients received postoperative irradiation and immunotherapy with autologous tumor cells emulsified in Freund's complete adjuvant and 10 patients received postoperative immunotherapy alone. During the 2-year course of this study, the 28-immunotherapy patients received between 4 and 10 serial immunizations with evidence of antitumor reactivity. Specifically, 25 of these patients developed cutaneous hypersensitivity responses to autologous tumor extracts with some sera samples showing cytotoxic antibodies against glioma cells in complement-dependent \textit{in vitro} assays. Bullard et al., (1984)\textsuperscript{799} conducted a trial on 5 patients, 3 of whom were given serial immunization with viable, HLA-mismatched glioma cell lines mixed with a BCG cell wall adjuvant in addition to standard chemotherapy of CCNU, Vincristine and procarbazine. Serological evidence of antitumor response without developing untoward system reactions was noted in two of the three serially immunized patients. At the sametime, Mahaley and coworkers\textsuperscript{702}, reported a phase I active immunotherapy trial in which
the same glioma cell lines (D54Mg and U251 Mg) were used to immunize serially 20 patients who were concomitantly treated with radiotherapy and BCNU chemotherapy postoperatively. Serological results of improved survival of patients receiving the U251Mg cell line compared to that of D54Mg cell line group was evident and no indication of untoward systemic reaction was reported. Other methods for improving the immunogenicity of tumor cells include modification of the cell membrane. Stavrou et al., (1981) chemically modified several different cell lines derived from rat MNU-induced gliomas with either trinitro-benzene sulphonic acid (TNBS) and dimethyl sulphate (DMS). Multiple immunization of syngeneic rats with this 'haptenized' glioma cells resulted in relatively specific cytotoxic antisera for unmodified glioma cells. Shinitzky and Skornick also modified tumor cells by incorporation of cholesteryl hemisuccinate (CHS) into the cell membrane. Immunotherapeutic vaccination of nude mice with irradiated, CHS-treated tumor cells afforded protection to subsequent challenges with unmodified viable tumor cells. This has so far, not attempted in patients with gliomas.

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* BCG: *Bacillus Calmette Geurin* is a bovine strain of *Mycobacterium tuberculosis*. 

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MOLECULAR THERAPIES:

The treatment of cancer is propelled by advances in the molecular biology of tumors and the unraveling of the cellular machinery. Molecular pharmacotherapeutic approaches to brain tumors can be broadly divided into gene therapy, antisense oligonucleotides, immunotherapy, and small molecules with inhibitory activity on tyrosine kinases, farnesyltransferase, and matrix metalloproteinases. The use of small molecules is designed to interfere with intracellular signal transduction by inhibiting one or several steps in the cell cycle, blocking angiogenic signals, or interfering with the ability of glioma cells to invade and spread.

Abnormalities in receptor tyrosine kinase pathways and loss of tumor suppressor genes are critical in the transformation and growth of malignant gliomas. Several workers have reviewed the molecular pathogenesis of gliomas in detail. Agents are available that selectively block steps in the Ras/MAPK/CDK/Rb cascade and that act on the PI3K/Akt pathway. In theory, every step could be inhibited, but in reality, the development of tyrosine kinase inhibitors has been most successful.

EGFR Tyrosine kinase inhibitors:

STI 571: (imatinib mesylate, Gleevec) is an oral phenylaminopyrimidine with inhibitory activity against platelet-derived growth factor receptor, c-kit, and the abl receptor tyrosine kinase. It inhibited the growth of U343 and U87 GBM cell lines in vitro and when implanted into the brains of nude mice. The North American Brain Tumor Consortium (NABTC) is testing STI 571 in a phase I and II trials in patients with recurrent malignant gliomas. STI 571 can cause several adverse side effects like thrombocytopenia, neutropenia, nausea, myalgias, oedema, diarrhoea, fatigue, skin rash and arthralgias.

ZD1839: is an anilinoquinazoline with a selective inhibitory action on the EGFR tyrosine kinase activity. Since overexpression of EGFR has been reported in 41% of GBM and is a probable negative prognostic factor in patients
under 60 years of age, ZD1839 is currently being investigated against malignant gliomas in several clinical trials. Diarrhoea, elevation of liver enzymes, acniform rash, nausea and vomiting were the most common toxicities.

**OSI-774**: (CP-358774 erlotinib, tarceva), another inhibitor of EGFR tyrosine kinase active at nanomolar concentrations as an ATP-mimic, blocks the cell cycle at G1, level of p27 and pRb accumulate and apoptosis is induced. An Active phase I trial of OSI-774 alone or in combination with temozolomide to treat high-grade gliomas with recurrent or stable disease provided good results. However, reported side effects include diarrhoea, fatigue, headache, mucositis, nausea, a transient elevation of liver enzymes and an acniform rash that can be severe.

**Phosphoinositide-3-kinase inhibitors:**

CCI-779 (Rapamycin analog drug):

Rapamycin is a macrolide produced by *Streptomyces hygroscopicus*, structurally related to cyclosporine and tacrolimus. Rapamycin and its analog CCI-779 inhibit a kinase known as mammalian target rapamycin, an enzyme activated through the PI3K/Akt cascades. This blockade leads to cell arrest in G1. Both these agents are cytostatic against xenografts of glioblastoma, medulloblastoma, breast cancer, PTEN-negative tumors seem to be more sensitive to inhibition with reduction of proliferation, tumor size and p70/56 kinase activity. Buckner et al., (2002) reported on a phase I study, where more than 50% of patients had stable disease and 38% received more than 9 courses. The most frequent symptoms are mucositis, skin rash, neutropenia and thrombocytopenia.
Review of Literature

Inhibition of Ras: Farnesyl Transferase inhibitors:

Ras is low-molecular weight GDP/GTP binding guanine triphosphatase (GTPase) with a determinant role in malignant transformation, invasion and spread of gliomas. Ras mutations or constitutive activation have been described in gliomas\(^{636}\), although controversy surrounds the observation of a mutated Ras gene \(^{640}\). Ras undergoes a series of post-translational modifications, starting with a lipid modification called farnesylation, which is catalyzed by farnesyl transferase (FTase) and depends on the enzymatic recognition of a specific carboxyl terminal sequence known as CAAX where C is cysteine, AA is aliphatic amino acid and X is any aminoacid, preferably methionine or serine. This process anchors Ras to the cell membrane, a required step of the cancer-causing activity of Ras.

1. **SCH66336**: (ionofarnib, Sarasar) is a 11 pyperidinyl trihalogenated, nonpeptidomimetic inhibitor of FTase and is active against tumors with wild type mutant Ras proteins \(^{816, 821}\). Cells exposed to SCH66336 can arrest in G1 or in G2/M. This agent inhibited viability and growth of glioblastoma cell lines U-251 Mg, U-251/E4 Mg and U-87 Mg in a time-and dose dependent manner \(^{822}\). Several clinical trials are underway but results are awaited\(^{823}\). Common toxicity effects include neutropenia, fatigue, nausea, vomiting, diarrhoea and neuropathy. An interesting effect of this agent is its ability to inhibit MDR 1 product p-glycoprotein, a protein with a major role in the development of resistance to chemotherapy by cancer cells\(^{824}\).

2. **R115777**: (Tipifarnib) is a nonpeptidomimetic methyl-quinolane inhibitor of FTase. It is a radiosensitizer, inducing postmitotic necrotic cell death in radio-resistant glioma cell line in vitro \(^{821, 825}\).

Several other inhibitors of vascular endothelial growth factor (VEGF) tyrosine kinase (SU5416 and PTK 787), matrix metalloproteinase (Marimastat, Metastat, Prinomastat), were also reported \(^{638}\).
The great potential benefit of these new treatments offers hope for significant improvement in the prognosis for patients with primary brain tumors, but the associated adverse side effects pose a threat to the patient's quality of life. Furthermore, new strategies are needed to determine the optimal dosing and effectiveness of these agents by evaluating the molecular effects as the endpoint rather than the traditional maximally tolerated dose and/or overall tumor response.

**IMMUNOTHERAPY WITH SHEEP RED BLOOD CELLS (SRBC):**

Sheep erythrocytes, long have been used as a classical antigen is a non-specific biological response modifier and exerts a strong immunomodulatory and antitumor property in experimental animals. SRBC are capable of forming spontaneous rosettes (E-rosettes) with T-lymphocytes, enhance T-lymphocytes proliferation and also augment IFN-γ production by lymphocytes. The 'active component' or the 'immuno-dominant' group responsible for its immunomodulatory and anti-tumor activity was found to be a cell surface glycoprotein molecule, termed "T11-target structure" (T11TS), or sheep from of LFA-3 (S-LFA3), or more currently CD58 that binds with the T11 (CD2) molecule present on the T-lymphocyte.

**Isolation and purification of T11TS:**

The T11TS (LFA-3) was isolated from the cell membrane of sheep erythrocyte by trypsin-digestion followed by ion-exchange chromatography on a DEAE cellulose column, which has been previously equilibrated with 0.05M formate buffer, pH6.8. The acidic glycopeptide was then eluted with a five chamber gradient system.

Studies have shown that the functional cross reactivity (CD2-T11TS) is most likely due to a structural homology of these molecules. In a study, a rabbit antiserum to sheep T11TS has been shown to cross react with LFA-3 (lymphocyte function associated antigen-3) in several independent assays: a) rabbit anti-T11TS antiserum has been shown to block Erythrocyte-rosette (E-rosette) formation by human T cells...
with both autologous and xenogenic erythrocyte by binding to the respective erythrocyte, b) the antisera has been shown to block the binding of anti-LFA-3 monoclonal antibody to human erythrocyte and c) it has been shown to react with purified LFA-3 in western blotting. Together, these findings have demonstrated that T11TS on sheep erythrocyte and LFA-3 (CD-58) on human erythrocyte are serologically related, which has provided further support for the notion that T11TS and LFA-3 (CD58) are the sheep and human forms of the same cell interaction molecule. Furthermore, NMR studies have shown that they have got 50% sequence homology and the electrostatic potential map of both binding surface are nearly identical.

The LFA-3 (CD58)/CD2 accessory pathway, has extensively been characterized in terms of structure and function of the CD2 molecule, which is present on all T lymphocytes and natural killer cells of the human, as well as rat immune system, also currently reported to be present on Macrophage, PMN and microglial cells. CD58 belongs to the immunoglobulin supergene family and is a membrane bound glycoprotein. The cDNA for human LFA-3 has been isolated. The cDNA defines a mature protein of 222 aminoacids that structurally resembles typical anchored proteins. Hydrophobic putative transmembrane region and a short cytoplasmic domain follow an extracellular domain with 6 N-linked glycosylation sites. The mature glycoprotein is estimated to be 44-68% carbohydrates. Northern blot analysis indicates that the LFA-3 mRNA of 1.3 kb is widely distributed in human tissue and cell lines. CD48 is the analogue of CD58 in rats and mice. However, CD48 and CD58 are structurally very similar, both are glycolipid-anchored molecules with two Ig-like domains and N-linked glycosylation sites (five and six sites respectively).

LFA-3 has been reported to have several isoforms. A transmembrane (TM) form and a glycosyl phosphatidylinositol (GPI)-linked form, differs only in their membrane anchoring mechanism, while a third isoform, LFA-3 delta D2, has a cytoplasmic tail of TM-LFA-3 but a truncated extracellular domain. It has been reported that LFA-3 delta D2 isoform, identified by RT-PCR analysis and DNA
sequencing, is also present in vivo like the other two isoforms, and shares a signal sequence with the TM and GPI isoforms.

Currently, NMR studies have provided structural information and support ‘hand-shake’ model of CD2-CD58 interaction involving the CFCC'C" faces of both CD2 and CD58 adhesion domains. This region responsible for binding specificity is most likely to be localized on the C, C' and C'-C'" loops on CD58. Osborn et al., (1995) have aligned the aminoacid sequence of LFA-3 and CD2 and mutagenised selected amino acids in the first domain of LFA-3 that are analogous to those implicated in the binding site of CD2. The CD2-CD58 crystal structure offers a detailed view of this key functional epitope: CD2 D31 and D32 orient the side-chain of CD58 K34 such that CD2 Y86 makes hydrophobic contact with the extended aliphatic component CD58 K34 between CD2 Y86 and CD58 F46. The elucidation of this ‘hot spot’ provides a new target for rational design for immunotherapeutic approaches.

Three distinct epitopes of the CD58 (LFA-3) molecules has been detected: T11(1), the SRBC binding site expressed on all T lymphocytes and thymocytes, T11(2), an epitope unrelated to the SRBC binding and T11(3), a neo-epitope expressed only upon T cell activation.

The binding of CD58 to CD2 facilitates the adhesion between T-lymphocytes and antigen presenting cells, and between cytolytic T cells, natural killer cells and their target cells. In addition, specific interactions between CD58 and CD2 have been shown to play an important role via a CD2 mediated co-stimulatory pathway during T-cell activation and proliferation. CD58-CD2 interaction can also reverse T cell anergy. CD58-CD2 interaction is also involved in the antigen-specific T cell activation, amplifying the signal generated by the T-cell receptor (TCR)-CD3 complex interacting with antigens. The role of LFA-3 in ‘antigen-dependent’ stimulation has been studied using antigen-reactive murine T cell hybridomas expressing human CD2. Expression of CD2 enhanced IL-2 production compared to the parent cell line in response to LFA-3+ antigen bearing cell JY, and this enhanced stimulation was inhibited by antibodies against CD2 or LFA-3. Hybridomas expressing region I or region II point mutation of CD2, which were
unable to bind LFA-3, did not exhibit an enhanced response to JY, while the wild type CD2+ hybridomas were able to produce IL-2 upon stimulation with liposomes, containing both purified LFA-3 and HLA-DR. T-cell activation via the CD2 pathway is also important mechanism for intrathymic T cell proliferation. Denning et al., (1988) have reported that thymocyte proliferation can be induced by purified LFA-3 in the presence of a region III anti-CD2 mAb and exogenous IL-2. Several other investigators have also proposed the role of CD2 pathway in T cell activation functions as an early thymocyte proliferative signal. This stimulation of T cell by LFA-3-CD2 interaction requires the presence of a second signal. Bierer (1988), Tiefenthaler (1987) and coworkers have assumed that this second signal may be provided by an antigen, an anti-CD3 mAb, or an anti-CD2 mAb direct to the region III epitope. However, in the thymus, the nature of this second signal is unknown. Bernard et al., (1997) have reported that for CD2 mediated costimulation of T cell receptor (TCR)-negative cells, two zeta-chain derived ITAM are sufficient to induce IL-2 when the CD2 molecules were co-cross linked with the chimeric CD25-zeta molecules, showing that the CD2 induced signaling does not necessarily employ the zeta chain in TCR-positive cells and that CD2 dependent costimulation in TCR-negative cells can be mediated via two functional zeta-chain derived ITAM. Cross linking of CD2 might activate T cells also in vivo via the src-like kinase p56 in concert with p62dok, thereby circumventing the requirement for other TCR associated signaling molecules like ZAP 70 kinase.

LFA-3 can costimulate cytokine secretion by cytotoxic T lymphocytes. Para et al., (1997) have observed that B7-1 (CD80) and LFA-3 costimulation activate CD8+ T cells to proliferate and secrete cytokines (IL-2, TNF-α, IFN-γ) and also cytotoxicity. Furthermore, CD58-CD2 interaction also optimizes immune recognition and facilitates contacts between T cells and APC. Lopez et al., (2001) have shown that activated monocytes provide a contact dependent factor (CD58/ LFA-3) and a soluble factor (IL-12) both critical for the in vitro expansion of CD56+ T cells. Sampaziotis et al., (2002) have reported that one perfect p53 (onco-suppressor protein) responsive elements is located in the first intron of the gene encoding for CD58 membrane protein.
The cerebral endothelial cells have the ability to express CD58 suggesting that by providing secondary signals to T cells proliferation within the CNS, it may be important in the initiation of inflammatory responses within the brain compartment\textsuperscript{864}. It has also been found that LFA-3 mediated adhesion, like that of ICAM-1 is functionally important in the molecular pathology of inflammatory disease\textsuperscript{865}.

Though, several reports elucidate the structural and immunological roles of CD58 (LFA-3) molecules, strikingly, no reports on disease relevance or function of such molecule in intact animals were available, until our studies provided interesting results of T11TS (CD58) in experimentally induced brain tumor models\textsuperscript{842, 867-868}. 