SUMMARY & CONCLUSION
Gliomas are the most common primary neoplasms of the central nervous system. Multimodality treatment—combined resection, radiotherapy and chemotherapy has long been shown to improve survival of patients with these neoplasms. Unfortunately, however, despite numerous trials of experimental therapy, the prognosis has not changed significantly in the last two decades: median survival is approximately 11 months post-diagnosis and 5 to 6 months post-tumor recurrence. These unfortunate results have stimulated interest in experimental approaches in the treatment of these insidious neoplasms. Immunotherapy is a particularly appealing strategy because of its theoretical potential for tumor-specific cytotoxicity and agents known as Biological Response Modifiers (BRMs) are occupying an interesting position.

The brain’s partial ‘immunoprivilege’ status further adds to the dilemma of brain tumor patients. However, Microglia (Mg), the brain’s representative of the immune system plays a key role in the initiation of an inflammatory response in the brain. Mg rapidly transforms from a resting state to an activated morphology in response to a variety of disease states. Many of the effector properties of Mg can be attributed to the array of substances they secrete in response to stimuli, such as LPS, cytokines and chemokines. Furthermore, Mg has the ability to present antigen to T cells, migrate in response to chemotactic stimuli, and phagocytose cell debris. This thesis work focuses on the immuno-modulatory functions of Mg, with particular attention to receptor expression, and highlights their pivotal role in the CNS.

In the present course of investigation, Mg cell activation and immunophenotypic modulations and their functional correlations were assessed in tumor bearing animal models by using different specific (IL-2, IFN-γ) and non-specific BRMs (SRBC), either alone or in combination. Although the specific biological response modifiers (BRMs) like IL-2, IFN-γ have been shown to modulate the Mg cell to get an effective antitumor immune response, associated toxicities and detrimental side effects have posed severe limitations in their use for therapeutic purposes. Comparatively, SRBC has provided greater modulatory effect on Mg cell
functions. The role of this BRM was profoundly stimulatory and has demonstrated anti-tumor properties. The critical interaction that occurs between the ‘T11 target Structure’ (T11TS) of the SRBC membrane and T11/CD2 molecule of the immunocytes. The active component responsible for such action was a cell surface glycoprotein (35-37 kD) and was isolated from the SRBC membrane and subsequently used as an immunotherapeutic agent for further studies keeping in mind its human application. So the thesis work has been divided into two sections, where section I deals with the selection of the best modulator of Mg activation and section II includes the application of T11TS as a biomodulator of Mg activity.

Though all three BRMs applied either separately or in combination contributed to improve the CMI status and subsequent rejection of glioma, separate dose of SRBC and also the combination dose of BRMs proved to be the two best options against glioma. Sometimes SRBC alone exceeded the combined effects of BRMs, which was well corroborated by the survival and histological findings.

Ethyl N-Nitrosourea (ENU) proved to be the most potent inducer of experimental brain tumor as evidenced by the histological findings and also survival results. The mean survival of the animal was found to be affected by the neurocarcinogenic effect of ENU and the survival was significantly decreased in the animals concerned, when compared to normal control. IL-2 and IFN-γ and also combination dose of the BRMs improved the survival but the best effect was observed in the SRBC treated group, where the survival of the animal was brought almost near to the normal level.

Microglia cells were isolated by enzymatic digestion followed with Percoll and Nycodenz density gradient centrifugation (elutrician) and were characterized by GFAP negativity and positivity for CD-11b, CD4 and RT1B (MHC-Class II). 

Assessment of cellular architecture in our study, after 5 months following ENU administration showed characteristic features of oligodendroglioma grade IV
neoplasia, such as clusters of oligodendrogial cells ('honey comb'), mitotic features, giant cells and absence of intracellular spacing and degenerative fibrils. Different BRMs induced somewhat modulatory effect and reversion of the hyperproliferating oligodendrogial cells to normal feature was observed after SRBC administration, indicating the role of SRBC as a better anti-cancer agent in comparison to the other BRMs used.

At the cellular level, the PLN assay depicted increased antigen presenting capacity of Mg in comparison to MØ. Further, the BRMs could effectively upregulate the antigen presenting capacity of both the immunocytes. SRBC provided better modulation of the antigen presentation capacity of Mg cells, which was significantly greater than that of IL-2, IFN-γ and combined doses.

Phagocytic assay revealed very mild phagocytic capacity of Mg with no significant modulation with the application of IL-2, IFN-γ, SRBC either alone or in combination dose. In contrast, the phagocytic activity of MØ and PMN were found to be upregulated by the administration of one or the other BRMs used, with the best effect produced by the SRBC administered group.

Scanning Electron Microscopic (SEM) images of Mg cells has provided evidences for the alteration of cellular ultrastructural topography under tumor induced and therapeutic conditions. The retraction of the cytoplasmic extensions in ENU-induced group shows the gross changes in cellular morphology when compared to normal control, suggesting modulation of cellular morphology during tumor pathogenesis. Significant modulation of Mg cells following BRM administration indicated the plastic nature of the Mg cells. Following SRBC administration, extensive protrusion of the cytoplasmic projections and filopodias were observed, strengthening the assumption the SRBC could be a potent modulator of Mg cell function and hint at the possible expression and/or upregulation of cell surface molecules.
Flowcytometric analysis of CD25 and MHC-class II receptor provided interesting findings. For the first time the presence of an upregulated IL-2R (CD25) was observed on Mg cells. Further different subtypes of Mg cells were found, expressing either single (CD25+ or MHC-Class II+) receptor or both the receptor (CD25+ MHC-Class II+) providing a novel idea of subdividing Mg cell according to their phenotypic expression. IL-2, IFN-γ or the combined dose of the BRMs upregulated the receptor expression in one or the other Mg subtype, but SRBC provided better modulation of both the receptors on Mg cells.

The signal transduction by protein tyrosine kinase (PTK) activity was found not to be prominent in Mg. The present data clearly establishes that IL-2 successfully transduces signals involving tyrosine kinases, but IFN-γ fail to do so. The current observation also justifies importance of SRBC as a dual potentiator, where on one hand it stimulates IL-12 production orienting the lymphocytes to a Th1 type cytokine profile and concurrently on the other hand results in highest modulation of the activation marker CD25 (IL-2R), resulting in an auto-activation feedback loop, culminating in the boosting of the immune status of the tumor bearing animal models.

Therefore, it can be assumed that among the different BRMs used, SRBC can exert better anti-cancer property in tumor-bearing animals by modulating the Mg cell surface receptor expressions and effector functions. Moreover, the cost effectiveness of SRBC treatment is many times more advantageous than the other established BRM therapies. In addition, administration of 0.5ml (i.p.) of SRBC has not produced any untoward reactions or toxicity. The excellent result of SRBC in the first section of the study design, provided impetus to perform further studies with its immunodominant group, i.e., T11 target structure (T11TS), which is assumed to be responsible for such anti-tumor activity of SRBC. The possible mechanism is the CD2 receptor mediated signaling on the immunocytes, which binds to the T11TS membrane glycoprotein of SRBC and turn the suppressed CMI response ‘on’ for taming brain tumor.
Survival studies have established the anti-tumor role of T11TS. The survival increased significantly with the three consecutive doses of T11TS administration in a dose-dependent manner when compared to the ENU-animals and the 3rd dose brought the survival of the animal almost to the normal level.

SEM studies corroborated with the FACS findings, Mg cell forming rosettes (E-rosette) with SRBC molecule were evidenced. Clear anchorage of Mg cell and SRBC membrane were observed; secretion of cytokines and secretory molecules as a result of CD2-T11TS ligand formation was also observed. Further, morphometric results have shown the effect of T11TS on modulation/activation of the Mg cell. A significant reduction in size of Mg cell was observed due to the carcinogenic effect of ENU as compared to normal. T11TS administration increased the Mg cell size and a massive cell size was achieved with the second dose of T11TS confirming the activated state of the Mg cell. 3rd dose, however, restored the Mg size almost back to normal. The results corroborated with the receptor modulation showing the maximum activation of Mg cell with the 2nd dose of T11TS administration.

FACS analysis, further, identified CD2 (unreported Mg receptor) receptor on Mg, opening up a possibility to regulate their response directly by T11TS. Hence, Mg presenting CD2 can be modulated by T11TS and thereby express their CD25, MHC-Class II and CD4 molecules which in turn regulate the immune profile within the cranium through interaction with brain infiltrating lymphocytes. CD2 and CD25 receptors were first reported to be present on Mg cells and their expression modulated with different doses of T11TS. Highest receptor saturation and Mg cell activation was observed in 2nd dose of T11TS administration, thereby upregulating the Mg activation markers CD4 and CD25. Activation was related to antigen presentation through MHC-Class II expression with repeated doses of T11TS.

Similar upregulatory trends of antigen presentation (MHC-Class II) and activation (denoted by the receptors CD4 and CD25) of Mg in first two doses of T11TS and then a decline of these properties in third dose hints towards a pro-
anti-inflammatory cascade of reactions between Mg and brain infiltrating T-cells which is yet to confirm by assessing the cytokine profile. The study pointed out existence of new immunophenotypic subsets of Mg and hint towards the role of Mg in the mechanism of brain tumor regression by T11TS.

Finally, PI-FACS apoptotic studies showed that T11TS could not effectively induce apoptosis in Mg cells while a dose-dependent apoptosis was induced in brain tumor cells. Rather, T11TS induced dose-dependent increase in synthesis of Mg cells. So, T11TS increased the proliferation as well as modulation of Mg cell leading to a steady pool of Mg cell effective for anti-tumor immune response in tumor bearing animals.

Similar results were observed by BrdU cellular DNA quantification assay by ELISA. The Mg cells were found not to be significantly affected by the inducing effect of T11TS, whereas brain tumor cells underwent profound apoptosis under the effect of the three booster doses of T11TS. Here again the T11TS proved to have proliferating effect on Mg cells leading to regression of tumor load in the tumor bearing animals.

In conclusion, the present set of results illustrates the potential importance of Mg for brain tumor or rather most neuropathologies. It remains a challenge for the future to uncover Mg functions in normal brain, and hope that this special thesis will help in stimulating continued interest among neuroscientists to explore new frontiers. Moreover, the immunomodulatory effect exhibited by SRBC and specially T11TS, were found to be unique in that they have direct stimulatory effects on almost every cellular events of the “Immunological Orchestra”, generating the best effective anti-tumor immune response and modulation of Mg cell. Therefore, from the present course of investigations, SRBC and its epitopic molecule, T11TS has offered its potential to be a rational therapeutic adjunct against gliomas.