Title of the thesis: AN IMMUNOLOGICAL APPROACH TOWARDS THE FUNCTIONAL ACTIVATION OF MICROGLIA IN BRAIN WITH AND WITHOUT TUMOR: HINTS FOR THERAPEUTIC MODULATION.

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ABSTRACT

The glial tumors are ‘difficult to treat’ neoplasms, refractory to conventional treatments. It is now recognized that immunotherapy could provide an alternative to the existing therapeutic modules.

Microglial cells, the chief immunomodulatory cells of the brain, play a pivotal role against various neurodegenerative diseases. These cells were isolated by enzymatic digestion followed with density gradient elutrician and were characterized by GFAP negativity and CD1lb, CD4 and RT1B (MHC class II) positivity.

In the present study, microglial cell activation and immunophenotypic modulations and their functional correlations were assessed in ENU-induced tumor bearing Druckrey rats by using different specific (IL-2, IFN-γ) and non-specific (SRBC) biological response modifiers (BRMs). The anti-tumor property of SRBC provided impetus to isolate the active component (i.e. T11TS) and subsequently use it as an immunotherapeutic agent for further studies keeping in mind its human application.

Survival and histological results demonstrated the potent neuro-carcinogenic effect of ENU (N-N’ ethylnitrosourea) and modulatory effect of BRMs, specially SRBC in comparison to other BRMs. Further, FACS analysis demonstrated different subtypes of microglial cell population (CD25+, MHC II+, and CD25+ MHC II+) in the brain based on phenotypic
expression, which were down-regulated in tumor bearing animals and restored significantly with SRBC administration. SEM study depicted similar modulatory effect of SRBC on cellular morphology of microglia. At the cellular level also SRBC–treated animals exhibited increased antigen presenting capacity of microglial cells. However, no significant increase in the phagocytic activity of the microglial cells was observed. IFN-γ and SBRC–treated animals also showed a down-regulatory PTK activity for microglia suggesting some alternate pathway for the activation.

In the second section T11- Target Structure (T11TS), which interacts with the CD2 (T11) molecule, was used as a therapeutic agent to assess microglial activation and immunophenotypic modulation. SEM results showed rosette formation of microglia with SRBC, indicating the presence of CD2 on microglia, which was confirmed with FACS. MHC class II and CD2 single and double positive populations were found to be modulated with different doses of T11TS, with the highest receptor saturation observed in 2nd dose of T11TS administration, thereby upregulating the activation markers CD25 and CD4. The presence of CD2 and CD25 receptors on microglial cell was reported for the first time. However, no significant inducing effect of T11TS on apoptosis of microglial cell was evident as compared to brain tumor cells.

The repeated modulation of microglial cells by the BRMs (SRBC and specially T11TS) culminated in an effective anti-tumor immune response in tumor bearing animals. The immunomodulation of microglia reflected their functional alterations suggesting T11TS to be an immunotherapeutic agent for brain tumor abrogation.