

ISN-CAEN Funds: 1.A. Visit by the applicant to another laboratory

DEVELOPMENT OF A PEPTIDE INHIBITOR OF THE INTERACTION BETWEEN CD160 AND BTLA WITH HVEM IN ORDER TO INCREASE ANTITUMORAL IMMUNITY FOR BRAIN CANCER

BACKGROUND: Glioblastoma is the most common and aggressive primary brain tumor. Complete surgical removal of these tumors is almost impossible due to their highly invasive character. Moreover, glioma cells develop resistance to most of the conventional therapies. Both factors promote tumor recurrence, which results lethal for virtually all patients. The immunosuppressive tumor microenvironment affects the function of cytotoxic T lymphocytes. One way to increase antitumor immunity is by blocking co-inhibitor receptors. HVEM (Herpes Virus Entry Mediator) is a co-signaling molecule belonging to TNF/TNFR superfamily, which function depends on the ligand that binds it. CD160 and BTLA (B and T Lymphocytes Attenuator), from the Ig superfamily, activate a co-inhibitory signal when they bind to HVEM, which reduces lymphocytes activation. On the other hand, L α (lymphotoxin α) and LIGHT (lymphotoxin-like), members from the TNF/TNFR superfamily, bind to HVEM and send a co-stimulatory signal. Although HVEM exerts dual function, its dominant and not redundant function is immunosuppressive. HVEM has three cysteine-rich domains (CRD), with a fourth different CRD. CRD1 is necessary for the co-inhibitory binding of CD160 and BTLA, but it is not involved in the co-stimulatory binding of LIGHT. Our **hypothesis** is that blocking the interaction of HVEM-CDR1 with CD160 and BTLA will increase lymphocyte activation and improve antitumor immunity. The **main goal** of this project was to develop and characterize peptides that block the interaction between CD160 and BTLA with HVEM-CRD1 without affecting the interaction with LIGHT, so as to increase the immunity against glioblastoma.

ACHIEVEMENTS

Synthesis of inhibitory peptides.

Exp. 1.1. We synthesized overlapped peptides of 15 amino acids as potential inhibitors of BTLA and CD160, which sequences were corresponding to the HVEM sequence that interacts with CD160 and BTLA. Peptides were synthesized by the Merrifield method and their purity was evaluated by HPLC.

Exp. 1.2. The biomolecular interaction between the synthesized peptides and BTLA or CD160, versus different control proteins, was evaluated by thermophoresis using Nanotemper tech. It was also analyzed by sandwich ELISAs.

Assessment of the effect of selected peptides on the function of immune cell populations.

Exp. 2.1. We analyzed the effect of the peptides on T-cell function *in vitro*. Leucocytes were purified from mouse spleen, cultured and activated with SIINFEKL and OVA peptides or antibodies against CD3 and CD28, in the presence or absence of selected HVEM peptides. Different populations of T cells were evaluated, measuring proliferation and IFN- γ production by thymidine incorporation assay and ELISA, respectively. Among all the peptides we developed, two were considerably distinguished from the rest, HVEM-20 and 30, which boosted the expansion and activation of T cells.

Exp. 2.2. We next assessed the effect of HVEM-20 and 30 *in vivo* in transgenic OT1 and wild type C57Bl/6 mice that were immunized with SIINFEKL, AH1 or Trp2. HVEM peptides were administered systemically, every day for 1 week. After the euthanasia, lymphocytes were purified from the spleens and proliferation and IFN- γ or IL-2 production was assessed by thymidine incorporation or ELISA and ELISPOT, respectively. Our selected peptides greatly improved the immune response in the Trp-2 immunization model.

Exp. 2.3. The effect of the peptides on DC function was analyzed by co-cultures of DC and CD4 $^{+}$ or CD8 $^{+}$ T cells purified from mice spleen, in mixed lymphocyte reactions, in the

presence or absence of increasing concentrations of the synthesized peptides. The stimulation of T-cell activation by DCs was evaluated measuring proliferation and IFN- γ production. We were not able to find a direct effect of the peptides on the ability of DCs to activate lymphocytes, but further experiments are needed to elucidate it.

FUTURE PROSPECTS

Now that I am back in Argentina, we will evaluate the therapeutic efficacy of the two selected HVEM-CDR1 inhibitory peptides with best performance in mouse models of glioblastoma and breast cancer. The antitumor immunity induced by DC vaccines loaded with tumor antigens and activated with TLR agonists will be evaluated in mice bearing intracranial or breast tumors, together with daily systemic injections of HVEM peptides. We will evaluate the presence of specific antitumor T-cells in the spleen, proliferation, circulating antitumor antibodies and tumor and immune cells markers by IHQ, ELISA and flow cytometry. We will also monitor survival. Moreover, we will analyze potential secondary effects such as the development of adverse autoimmune lesions.

OUTCOMES AND BENEFITS

These four months working in CIMA allowed me to prove our hypothesis correct. We have developed peptides that inhibit the interaction of HVEM with CD160 and BTLA, which strongly stimulate the immune response. Our next goal is to continue with the evaluation of the therapeutic efficacy in the glioblastoma and breast cancer models that we have in our lab, which will consolidate the international collaboration between our group and Dr. Lasarte's laboratory. The goal of my PhD thesis is to develop strategies that neutralize the immunosuppressive tumor microenvironment and stimulate cytotoxic T lymphocytes for the treatment of Glioblastoma, without generating significant toxicity. This fellowship gave me the opportunity to obtain results of great impact on the development of my doctoral thesis, given the therapeutic potential of the HVEM peptides for the activation or blockade of molecules involved in the pathogenesis of glioblastoma. We expect to publish these results in a research paper in the near future. I am very happy for this experience and I would like to express my gratitude to the International Society of Neurochemistry for this opportunity.

FINANTIAL REPORT

The funds provided by the ISN-CAEN were used to partially cover travel and housing expenses.



