NEUROGENERATION AND NEUROPROTECTION BY COPOLYMER 1 AND THERAPEUTIC VACCINES

Department of
of Immunology

Prof. Ruth Arnon

Rina Aharoni , Michael Sela,
Nathalie Moyal-Amsellem

☎ 08-934 4017
FAX 08 9469712
@ Ruth.Arnon@weizmann.ac.il
www.weizmann.ac.il

A. Neurogenesis and Neuroprotection Induced by Glatiramer Acetate Treatment of EAE

In multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE), the immune system provokes the detrimental process via autoimmune inflammatory mechanisms. Still, neuronal and axonal degeneration, initiated at disease onset and revealed when compensatory CNS resources are exhausted, are the major determinant of the irreversible neurological disability, particularly in the myelin oligodendrocyte glycoprotein (MOG) induced model. Brain

insults such as the autoimmune inflammatory process in both diseases induce a measure of neurogenesis, but its regenerative therapeutic consequence is limited, as it fails to regenerate functional neurons and compensate the damage. Current treatments for MS are effective in ameliorating the immune inflammatory process, but their ability to enhance the intrinsic CNS repair mechanism and to induce effective neuroprotection and neurogenesis has not been shown. Glatiramer acetate (GA), an approved drug developed in our laboratory for MS treatment, exerts a marked suppressive effect on EAE induced by various encephalitogens, in several species (Arnon and Sela, 2003).

The immunomodulatory effect of GA was attributed to its ability to induce Th2/3 cells that secrete high levels of anti-inflammatory cytokines. These cells cross the blood brain barrier (BBB), accumulate in the CNS (Aharoni et al., 2002), and express in situ interleukin 10 (IL-10), transforming growth factor-β (TGF-β), as well as Brain Derived Neurotrophic Factor (BDNF) (Aharoni et al., 2003, 5005). We further investigated whether peripheral immunomodulatory treatment for by GA, can enhance neurogenesis and generate neuroprotection in the CNS of EAE inflicted mice. EAE was induced by myelin oligodendrocyte glycoprotein (MOG) peptide, either in YFP 2.2 transgenic mice, which selectively express YFP on their neuronal population or in C57BL/6 mice. The in situ effect of GA was studied in various brain regions; neuroprotection and neurogeneration were evaluated and quantified by measuring the expression of different neuronal antigens and in vivo proliferation markers. The results demonstrated that in EAE-inflicted mice neuroproliferation was initially elevated following disease appearance, but subsequently declined below that of naive mice. In contrast, GA treatment in various stages of the disease, led to sustained reduction in the neuronal/axonal damage typical to the neurodegenerative disease course. Moreover, three processes characteristic of neurogenesis, namely cell proliferation, migration and differentiation, were augmented and extended by GA treatment in EAE mice, in comparison to EAE untreated mice and naive controls. The newborn neuroprogenitors manifested massive migration through exciting and dormant migration pathways, into injury sites in brain regions, which do not normally undergo neurogenesis, and differentiated to mature neuronal phenotype. This suggest a direct linkage between immunomodulation, neurogenesis and an in situ therapeutic consequence in the CNS. Recent findings demonstrate that GA plays a role in the demyelination process, both in the prevention of demyelination and possibly leading to myelin repair.

Another autoimmune disorder in which GA exhibits a beneficial effect is Inflammatory Bowel Diseases (IBD), the experimental model for Crohn's disease. In these animal models, comprising acute and chronic IBD, daily treatment with GA led to a substantial suppression of the disease, manifested in reduced weight loss, increased survival, as well as prevention of histopathological damage in the gut.

B. Therapuetic and Prophylactic Effects on Tumor Growth of Recombinant Flagella

The flagellin of a Salmonella vaccine strain was found to be an adequate carrier of epitopes for vaccination against viral and bacterial agents. Immunization with the recombinant flagella expressing epitopes of various viral and bacterial pathogens was shown to evoke humoral as well as cellular immune responses against the inserted epitope, which resulted in protection against a challenge infection. However, this approach has never been applied to cancer therapy. To that end, the effect of a recombinant flagella carrying an epitope of the human mucine 1 (MUC1) on the growth of tumor expressing MUC1 was investigated. MUC1 is a large glycoprotein (>200 KDa) expressed on the apical surface of most of the glandular epithelia. In breast carcinoma, MUC1 is overexpressed on the full surface of the cell and its glycosylation groups are altered, which make this tumor associated antigen a good candidate for immunotherapy. Therefore, Balb/c mice bearing 4T1-MUC1 tumor (a mouse mammary carcinoma cell line modified to express the human MUC1) were used as an animal model of breast cancer. The results show that in a therapeutic vaccination experiment tumor- bearing mice immunized subcutaneously (s.c.) once with flagellin expressing MUC1 epitope displayed a 3 fold reduction in tumor size in comparison to the control group ($p<0.05$). In a prophylactic vaccination experiment, mice immunized (s.c.) 3 times at 4 weeks intervals with this construct in adjuvant exhibited a significant 6 times smaller tumor size than the control group ($p<0.05$). There are several indications implicating cellular immune response in this tumor reduction effect (lymphocyte proliferation), whereas humoral response is apparently not involved. These results indicate that recombinant flagellin might be a suitable carrier for a novel approach towards vaccination against cancer, leading

to both therapeutic and prophylactic effects. Two epitopes of MUC1 were employed MUC1.7 comprising 7 amino acids residues, known as a B- and T-cell epitope, and MUC1.9, comprising 9 residues, which exhibits the strongest binding affinity to MHC. Fla constructs of both epitopes showed efficacy in reducing the tumor growth and their mixture exhibited higher-therapeutic activity. An even higher efficacy was demonstrated by the construct Fla MUC1.25, which expressed the entire tandem repeat sequence with 5 additional residues.

Selected publications

Aharoni, R., Meshorer, M., Sela, M. and Arnon, R. (2002) Oral

treatment of mice with Copolymer 1 (Glatiramer Acetate)

results in the accumulation of specific TH2 cells in the central

nervous system. J. of Neuroimmunology, 126, 1-2.

Aharoni, R., Kayhan, B., Eilam, R., Sela, M. and Ruth Arnon.

(2003) Glatiramer acetate specific T-cells in the brain express TH2/3

Cytokines and brain-derived neurotrophic factor in situ PNAS, Vol

100, 24, 14157-14162.

Arnon, R. and Sela, M. (2003) Immunomodulation by the

Copolymer Glatiramer Acetate. J. of Molecular Recognition, 16,

412-421.

Aharoni, R. and Arnon, R. (2005) Neurogenesis and

Neuroprotection induced by peripheral immunodulatory

treatment of experimental autoimmune encephalomyelitis. J. of

Neuroscience. 25 (36), 8217-8228.

Aharoni, R., Eilam, R., Domev, H., Labunskay, G., Sela, M and

Arnon. R. (2005) The immunomodulator glatiramer acetate augements the expression of neurotrophic factors in brains of experimental autoimmune encephalomyelitis mice. PNAS, 52, 19045-19050.

Rina Aharoni, Hagar Sonego, Ori Brenner, Raya Eilam and Ruth Arnon (2007) The therapeutic effect of glatiramer acetate in a murine model of inflammatory bowel

disease is mediated by anti-inflammatory T-cells. *Immun. Letts.* **112:** (2): 110-119.

Nathalie Moyellem, Ruth Arnon (2008) Reduction of established tumors in mice by recombinant flagella-MUC1 based vaccine. (submitted).

Acknowledgements

Research Grants from the TEVA Company for Studies on GA

The Paul Ehrlich Chair in Immunology ; Mr. and Mrs. Eugene Applebaum Dr. and Mrs. Claude Oster.