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Glatiramer Acetate - Mechanism of Action in Multiple Sclerosis and Potential for New Applications

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The ability of a remedy to modulate the pathological process in the target organ is crucial for its therapeutic activity. Glatiramer acetate (GA Copaxone®, Copolymer 1) is an approved drug for the treatment of multiple sclerosis, and is highly effective in the suppression of experimental autoimmune encephalomyelitis in various species. The mode of action of GA is by initial strong promiscuous binding to most major histocompatibility complex (MHC) Class II molecules and the consequent competition with MBP and other myelin proteins for their binding and presentation to T cells. A further step of its mode of action is the potent induction of specific suppressor cells of the regulatory Th2 type. We investigated whether these GA-specific T cells can function as suppressor cells with therapeutic potential in the target organ by *in situ* expression of T helper 2/3 cytokines and neurotrophic factors. GA-specific cells and their *in situ* expression were detected on the level of whole-brain tissue by using a two-stage double-labeling system: (i) labeling of the GA-specific T cells, followed by their adoptive transfer, and (ii) detection of the secreted factors in the brain by immunohistological methods. GA-specific T cells in the CNS demonstrated expression of two anti- cytokines, IL-10 and transforming growth factor ß (TGF ß), whereas no expression of the inflammatory cytokine IFN-γ was observed (Fig 1A). This pattern of expression was manifested in brains of normal and experimental autoimmune encephalomyelitis-induced mice to which GA-specific cells were adoptively transferred, but not in control mice. Furthermore, infiltration of GA-induced cells to the brain resulted in bystander expression of IL-10 and TGF ß, by resident astrocytes and microglia (Fig 1B). The GA-specific cells in the CNS demonstrated also intense expression of brain-derived neurotrophic factor (BDNF), as shown in Fig. 1C). The ability of infiltrating GA-specific cells to express anti-inflammatory cytokines and neurotrophic factor in the organ in which the pathological processes occur correlates directly with the therapeutic activity of GA in EAE/MS. Based on this mode of action, we explored the potential of GA for two other applications – prevention of graft rejection and amelioration of inflammatory bowel diseases (IBD).

Concerning graft rejection, we have previously demonstrated that GA suppresses the immune rejection manifested in graft versus host disease as well as in graft rejection. In an attempt to reduce the dosage and toxicity of the current immunosuppressive regimens, we have now tested the ability of GA, combined with low doses of cyclosporin (CyA) or tacrolimus (FK506), to suppress the rejection of strongly mismatched allografts across major histocompatibility barriers. Recently we showed that such combination therapy was affective in several animal models: 1) It led to a significant delay of the vigorous process of skin rejection in mice, manifested by 2.1-5.4 fold prolongation in skin graft survival. 2) The combined treatment led to efficient inhibition of the functional deterioration of thyroid grafts in mice, manifested by 2.2-20.1 fold increase in iodine absorbance of the transplanted thyroids, as compared to each drug alone. 3) Combination therapy inhibited significantly the rejection of vascularised heart transplants in rats, a system that is more closely relevant to organ transplantation in patients. Thus, cardiac allograft survival following the combined treatment with GA and low dose of CyA was longer than the survival obtained by four fold higher dose of CyA alone. In all transplantation systems combination therapy of GA with either CyA or FK 506 significantly suppressed graft rejection, and was more effective than treatment with either GA or the immunosuppressive drug alone, suggesting that such treatment may be

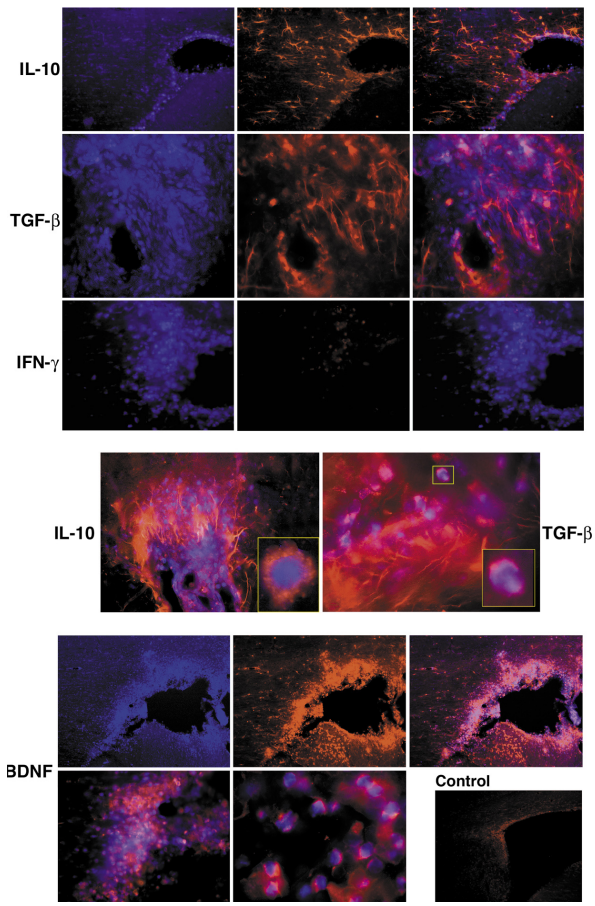

Fig. 1 *Immunohistochemical analysis of cytokines and BDNF expression by GA-specific cells in the brain. Activated Hoechst labeled GA-specific cells were injected into the peritoneum of EAE induced mice. After seven days, the mice were perfused and brain sections (20μ) were stained immunocytochemically for IL-10, TGF-β, INF-α and BDNF. A. Left column – staining for Hoechst labeling (blue), Middle column – immunohistological staining of the specific cytokines (red). Right column - merged images. B. Merged images of sections depicted Perivascular infiltrations in the cortex. Enlarged regions accentuate the complete overlap on a single cell level. C. Staining for BDNF. Top - an area surrounding the lateral ventricle. Bottom, left – merged image of perivascular area. Middle – enlarged section merged image of single cell staining. Right – Brain section from a control EAE induced mouse that did not receive GA-labeled cells, stained for BDNF. Scale bar for A and C-bottom left is 50μm, B 20μm, C-top line and control 100μm.*

beneficial for human transplantation.

Inflammatory bowel diseases (IBD) are also characterized by detrimental immune reactivity in the gut and imbalance between pro-inflammatory and anti-inflammatory reactivity. In an attempt to down regulate IBD we tested whether glatiramer acetate can ameliorate trinitrobenzene sulfonic acid (TNBS)-induced colitis – a murine model that resembles human Crohn's disease (CD). Experimental colitis was induced by rectal instillation of TNBS in three mice strains: BALB/c, SJL/J, and (SJL/JXBALB/c)F1, and its severity was evaluated by gross colon injury, histological damage, body weight and survival rate. We studied the effect of GA on all these parameters as well as on lymphocyte reactivity manifested by proliferation and secretion of tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β). We demonstrated that GA treatment significantly suppressed the various manifestations of TNBS-induced colitis as demonstrated by substantial reduction in the macroscopic colonic damage, preservation of the microscopic colonic structure, reduced weight loss, and improved long-term survival, in GA treated mice compared to untreated mice. Parenteral route was more effective than oral. GA suppressed the proliferation of local mesenteric lymphocytes in response to syngeneic colon extract and the detrimental TNF-α secretion. In addition, it induced a beneficial secretion of TGF-β, indicating again the involvement of a TH1 to TH2 shift as part of the GA mode of action.

Selected Publications

1. R. Aharoni, D. Teitelbaum, M. Sela and R. Arnon. J. of Neuroimmunology. 91, 135-146 (1998).
2. R. Aharoni, B. Kayhan, R. Eilam, M. Sela and R. Arnon. PNAS. Vol. 100: 24: 14157-14162 (2003)
3. R. Aharoni, D. Teitelbaum, R. Arnon and M. Sela. Transplantation, 72: No 4, 598-605 (2001)
4. R. Aharoni, A. Yussim, M. Sela and R. Arnon. Int. Immunopharmacology. In press. (2004).

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