Our main objectives are to shed light on the mechanisms underlying the dysregulation of the immune system in two autoimmune diseases, namely systemic lupus erythematosus (SLE) and myasthenia gravis (MG), and to develop novel specific therapeutic means for the treatment of these diseases.

**Myasthenia gravis (MG)** (in collaboration with Michael Sela)

MG is a T cell regulated, antibody mediated autoimmune disease. Two peptides representing sequences of the human acetylcholine receptor α-subunit were shown to be immunodominant T cell epitopes in MG patients as well as in inbred mouse strains. A dual analog composed of the tandemly arranged single analogs of the two myasthenogenic peptides inhibited in vitro and in vivo, MG associated autoimmune responses. Further, treatment with the dual analog down regulated the clinical manifestations of an ongoing experimental autoimmune MG (EAMG). The beneficial effects on the clinical disease correlated with a reduced production of anti-acetylcholine receptor autoantibodies and with a dramatic decrease in the secretion of the pathogenic cytokine INF-γ. Thus, the dual analog is an efficient immunomodulator of EAMG in mice (Fig. 1) and might be of specific therapeutic potential for MG.

The inhibitory effect of MG associated responses by the dual analog has been also demonstrated using peripheral blood lymphocytes of patients with MG. Thus the dual analog inhibited efficiently the in vitro proliferative responses of the latter cells to the native acetylcholine receptor. The inhibition was associated with a significant decrease in the secretion of the pathogenic cytokine INF-γ and with an increased production of the immunosuppressive cytokine TGF-β (Fig. 1).

Attempts to elucidate the mechanism(s) by which the dual analog immunomodulates MG associated autoimmune responses indicated that treatment with the dual analog down regulated the production of the Th1-type cytokines, IL-2 and IFN-γ (known to play a pathogenic role in MG and EAMG) and up regulated the secretion of the immunosuppressive cytokine TGF-β. Further, the immunosuppressive effect could be transferred into naïve mice by their inoculation with splenocytes of dual analog treated mice (Fig. 1).

Immunization of mice with a myasthenogenic peptide increased the adhesiveness of lymph node derived T cells to VCAM-1, expanded the population of lymph node or spleen derived T cells expressing functional receptors for P- and E- selectin, enhanced the secreted levels of MMP-9, and led to an increase in TCR-associated PLC activity. In vivo administration of the dual analog inhibited all above peptide induced processes. Thus, the ability of the dual analog to interfere with signaling and migration associated events (Fig. 1) results in its significant therapeutic potential.

**Systemic lupus erythematosus (SLE)**

SLE is an autoimmune disease characterized by an increased production of autoantibodies, impairment of B and T cell functions, and systemic clinical manifestations. Experimental SLE can be induced in mice (not SLE-prone) by immunization with the human anti-DNA monoclonal antibody that bears the common idiotype, 16/6 Id. Autoantibodies of the diseased mice were shown to be highly homologous to anti-DNA monoclonal antibodies isolated from SLE-prone (NZB×NZW)F1, MRL/lpr/lpr) mice. Two peptides based on the complementarity determining regions (CDR) 1 and 3 of pathogenic murine and human monoclonal anti-DNA antibodies with the 16/6 Id were synthesized and shown to prevent autoantibody production, T cell activation and SLE associated manifestations in mice. The CDR based peptides were capable of either preventing
or treating an established disease that was induced with the human anti-DNA 16/6Id or developed spontaneously in (NZBxNZW)F1 mice. The beneficial effects were manifested in a reduction of anti-dsDNA autoantibody levels and amelioration of kidney damage (Fig. 2). The latter also correlated with an immunomodulation of the proinflammatory cytokines and of the Th1 and Th2-type cytokines, and with a significant upregulated secretion of the immunosuppressive cytokine TGF-β (Fig. 2). Further, the immunosuppressive effect could be transferred by splenocytes of mice treated with the CDR based peptides into recipients with full blown disease.

The ability of the CDR based peptides to inhibit the 16/6Id specific proliferation of peripheral blood lymphocytes of SLE patients was also assessed. The peptides based on the CDR1 and CDR3 of the autoantibodies of murine and human origin inhibited efficiently and specifically the proliferation. The inhibition correlated with a reduction in IL-2 secretion and with an up-regulated production of the immunosuppressive cytokine, TGF-β. Thus, these peptides are potential candidates for a novel specific treatment of SLE patients.

Matrix metalloproteinases (MMPs) constitute a family of zinc containing endo-proteinases. We have demonstrated that MMP-9 activity is significantly elevated in sera of SLE patients as compared to healthy controls. We have also shown that levels of both MMP-3 and MMP-9 are elevated in sera and in kidneys of mice afflicted with either the spontaneous or the induced experimental SLE. Treatment of the SLE afflicted mice with the CDR based peptides, diminished the levels of these MMPs in the serum (Fig. 2) and kidneys of the treated mice. The results suggest that MMP-3 and MMP-9 play a pathogenic role in SLE and that these enzymes may serve as markers for the determination of disease progression or amelioration.

Selected Publications

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