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# Specific Immunomodulation of autoimmune diseases

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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 their treatment.

### Myasthenia Gravis (MG) (in collaboration with Michael Sela)

MG and experimental autoimmune MG (EAMG) are T-cell regulated, antibody mediated diseases. Two peptides, representing sequences of the human acetylcholine receptor (AChR)  $\alpha$ -subunit were shown to be immunodominant T cell epitopes in MG patients as well as in inbred mouse strains. A dual altered peptide ligand (APL) composed of the tandemly arranged two single amino acid analogs of the two myasthenogenic peptides inhibited *in vitro* and *in vivo* MG associated autoimmune responses. Furthermore, the dual APL down-regulated the clinical manifestations of established EAMG induced either in mice or rats by the Torpedo AChR. The dual APL inhibited also the proliferative responses of peripheral blood lymphocytes (PBL) of MG patients to the native AChR. Thus, the dual APL is a candidate for the specific immunotherapy of MG.

The *in vitro* and *in vivo* inhibiting effects of the dual APL were associated with a significant down regulation of the pathogenic  $\text{IFN}\gamma$  and with an up-regulation of the immunosuppressive cytokine,  $\text{TGF}\beta$ . Furthermore, the suppressive effect of the dual APL could be transferred to mice, immunized with either the myasthenogenic peptides or with the AChR, by their inoculation with immunocytes of dual APL treated mice. Administration of the dual APL was shown to activate CD4CD25 expressing cells. Furthermore, depletion of these cells diminished the specific inhibitory effect of the dual APL. Treatment with the dual APL also down regulated the CD28 activating molecule and up regulated the inhibitory CTLA-4 molecule. Thus, CD4<sup>+</sup>CD25<sup>+</sup> immunoregulatory cells play a key role in the inhibitory effects of the dual APL, either directly through the CTLA-4 molecule expressed on these cells, and/or indirectly by causing the differentiation of other regulatory T cell population/s that secrete  $\text{TGF}\beta$ .

Further characterization of the CD4<sup>+</sup>CD25<sup>+</sup> cells, demonstrated an up-regulated expression of both CTLA-4 as well as intracellular and membranal  $\text{TGF}\beta$  in the dual APL treated mice. Investigation of signaling events induced by the dual APL, showed an association between  $\text{TGF}\beta$  levels and JNK activity. JNK protein is known to activate the transcription of the FasL gene that upon binding to Fas receptor initiates activation induced cell death. As expected treatment with the dual APL increased the apoptotic rate as measured by the expression of Fas, FasL and phosphatidylserine exposure (Figure 1). Thus, the ability of the dual APL to interfere with signaling associated events results in its significant therapeutic potential.

### Systemic lupus erythematosus (SLE)

SLE is an autoimmune disease characterized by an increased production of autoantibodies and systemic

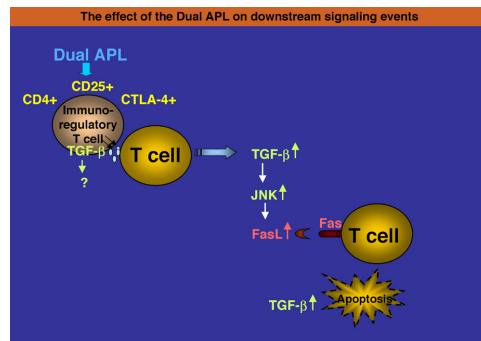


Fig. 1

clinical manifestations. For a specific treatment of SLE we have designed and synthesized a peptide (hCDR1) based on the complementarity determining region (CDR) 1 of an anti-DNA autoantibody and tested its effects on several mice models for lupus: a) the spontaneous model of (NZBxNZW)F1 mice, b) induced experimental SLE in BALB/c mice, and c) a "humanized" model of severe combined immunodeficient (SCID) mice engrafted with PBL of SLE patients. Treatment with hCDR1 ameliorated the serological and clinical manifestation of the established lupus in these models. The beneficial effects of hCDR1 were associated with a decreased secretion and expression of the pathogenic cytokines,  $\text{IFN}\gamma$ , IL-10, IL-1 $\beta$  and TNF $\alpha$  and with an up-regulation of the immunosuppressive cytokine, TGF $\beta$ . Further, treatment with hCDR1 reduced apoptosis rates (TUNEL, Fas/FasL) that were elevated in the SLE afflicted mice.

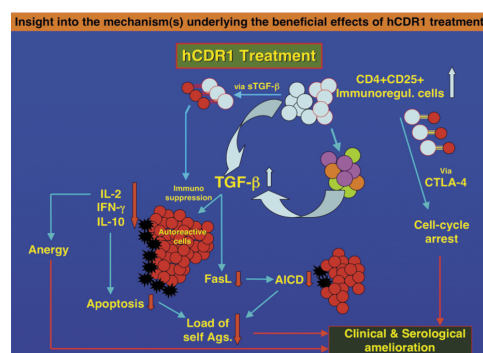


Fig. 2

For a better insight into the mechanism(s) underlying the inhibitory effects of hCDR1 we characterized splenocytes of young mice that were treated with hCDR1. Injection of hCDR1 up-regulated the CD4CD25-expressing cells that were also determined to be CD45RB<sup>low</sup> and CTLA4<sup>+</sup>, and to express elevated levels of intracellular and membranal TGF $\beta$ . Adoptive transfer of hCDR1-treated splenocytes to old (NZBxNZW)F1 mice with a full blown disease resulted in a reduction of the anti-dsDNA autoantibodies and in the amelioration of the kidney disease in the recipient mice. Depletion of the CD25<sup>+</sup> cells diminished significantly the therapeutic effects of hCDR1, whereas administration of the enriched CD4<sup>+</sup>CD25<sup>+</sup> cell population was beneficial to the diseased mice. We therefore suggest that treatment with hCDR1 results in CD4<sup>+</sup>CD25<sup>+</sup> cell expansion that may cause the suppression of autoreactive cells by cell-cell

interaction via surface TGF $\beta$ , and/or cell cycle arrest through CTLA-4. Further, these immunoregulatory cells trigger directly or indirectly, the secretion of TGF $\beta$ , thus contributing to the immunosuppression of autoreactive cells and to the amelioration of SLE (Figure 2).

We have further shown (with Ofer Lider) that administration of hCDR1 diminished the adhesive and chemotactic responses to SDF-1 $\alpha$  of autoreactive T cells. The inhibitory response, correlated with ERK phosphorylation and was associated with increased secretion of TGF $\beta$ .

In view of its demonstrated beneficial effects the hCDR1 is a candidate for a novel specific treatment of SLE patients.

### Selected Publications

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- Zinger H, Eilat E, Meshorer A, Mozes E (2002) Peptides based on the complementarity-determining regions of a pathogenic autoantibody mitigate lupus manifestations of (NZBxNZW)F1 mice via active suppression. *Int Immunol* 15: 205-214
- Paas-Rosner M, Sela M, Mozes E (2003) A dual altered peptide ligand down-regulates myasthenogenic T cell responses by up-regulating CD25- and CTLA-4 expressing CD4<sup>+</sup> T cells. *PNAS* 100: 6676-6681
- Stoeger ZM, Dayan M, Tcherniack L, Green L, Toledo S, Segal R, Elkayam O, Mozes E (2003) Modulation of autoreactive responses of peripheral blood lymphocytes of patients with systemic lupus erythematosus by peptides based on human and murine anti-DNA autoantibodies. *Clin Exp Immunol* 131: 385-392
- Dayan M, Stoeger Z, Neiman A, Abarbanel J, Sela M, Mozes E (2004) Immunomodulation by a dual altered peptide ligand (APL) of autoreactive responses to the acetylcholine receptor of peripheral blood lymphocytes of patients with myasthenia gravis. *Hum Immunol* (In press)

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