Specific Immunomodulation of Autoimmune Diseases

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Our main objectives are to shed light on the mechanisms underlying the amelioration of two autoimmune diseases, namely, myasthenia gravis (MG) and systemic lupus erythematosus (SLE), by peptides that we designed as novel means for the specific treatment of these diseases.

Myasthenia gravis (MG)

Myasthenia gravis (MG) and its experimental model, experimental autoimmune MG (EAMG), are immune disorders that are characterized by circulating antibodies and lymphocyte autoreactivity to the nicotinic acetylcholine receptor (AChR), leading to a reduced number of AChR molecules at the postsynaptic end plates. Two myasthenogenic peptides, p195-212 and p259-271, that are sequences of the human AChR α-subunit were shown to induce MG-associated immune responses in mice. A dual altered peptide ligand (APL) composed of the two single amino acids substituted analogs of the two myasthenogenic peptides inhibited *in vitro* and *in vivo* MG-associated responses, and it down-regulated the clinical manifestations of an established EAMG induced either in mice or in rats by the Torpedo AChR. The inhibiting effects of the dual APL were associated with a significant down-regulation of the pathogenic cytokine IFN-γ and with an up-regulation of TGF-β and IL-10.

The dual APL acts by up-regulating $CD4^+CD25^+$ cells that express characteristic regulatory markers such as $CD45RB^{low}$, Foxp3, CTLA-4 and intracellular and membrane-bound TGF-β. Further studies demonstrated that CD8 regulatory cells are also involved in the suppressive function of the dual APL although their effects were less prominent than those of $CD4^+CD25^+Foxp3^+$ regulatory cells. Treatment with the dual APL increased the apoptotic rate of autoreactive cells as measured by the up-regulation of the key markers Fas, FasL, caspases 3 and 8 and by the down-regulation of the anti-apoptotic markers cFLIP and Bcl-2.

Furthermore, the dual APL was shown to up-regulate the phosphorylation of ERK1,2 in $CD4^+CD25^+$ cells, whereas it decreased the phosphorylation of ERK1,2 in $CD4^+CD25^-$ cells. Inhibition of ERK1,2 in the dual APL induced $CD4^+CD25^+$ cells, abrogated their ability to suppress MG-associated responses. In addition, the dual APL up-regulated a novel 50 kDa ERK in the induced $CD4^+CD25^+$ cell population. Thus, the ability of the dual APL to interfere with signaling associated events results in its significant therapeutic potential.

Systemic lupus erythematosus (SLE)

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by an increased production of autoantibodies and systemic clinical manifestations. T and B cells play an important role in disease development. 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. Treatment with hCDR1 ameliorated the serological and kidney-related manifestations of the established lupus in these models. Further, hCDR1 down-regulated disease manifestations associated with neurological lupus as demonstrated by reduced brain pathology and improved behavior parameters (e.g. anxiety, memory). The beneficial effects of hCDR1 were associated with a decreased secretion and expression of the pathogenic cytokines, IFN-γ, IL-10, IL-1β and TNF-α and with an up-regulation of the immunosuppressive cytokine, TGF-β.

hCDR1 was shown to down-regulate the secretion and expression of BAFF (BLyS), a crucial regulator of B cell development, maturation and survival. The diminished production of BAFF was associated with an improvement in the clinical manifestations of lupus.

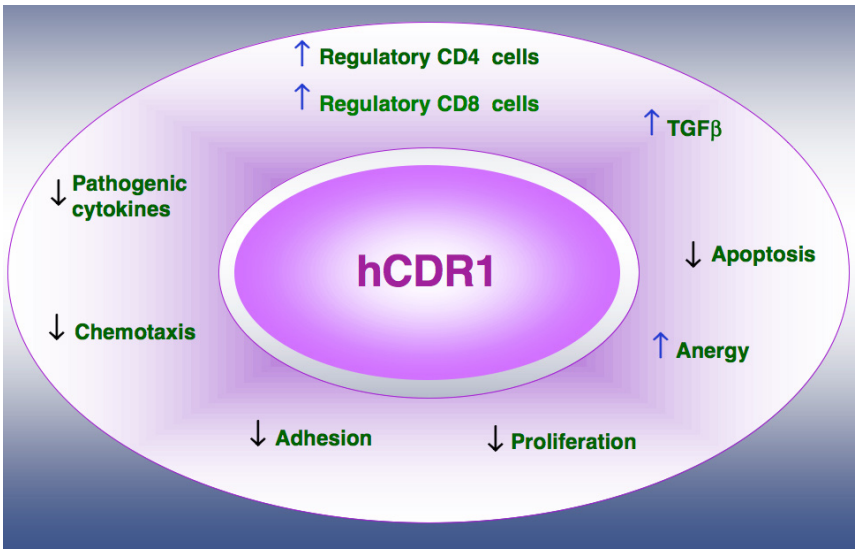

Fig. 1 *Mechanisms of action of hCDR1.*

Treatment with hCDR1 up-regulated the $CD4^+CD25^+CD45RB^{low}$ cells with regulatory characteristics, including the expression of Foxp3, CTLA-4 and TGF-β (Figure 1). The hCDR1-induced CD4CD25 expressing cells suppressed the activation of autoreactive $CD4^+$ cells as indicated by the diminished

expression of CD69 and Fas ligand on the latter, resulting in reduced rates of activation-induced apoptosis. The latter was also indicated by the reduced expression of caspases 3 and 8 and by the up-regulation of the anti apoptotic molecule Bcl-xL, which had tolerogenic effects on SLE. In addition, treatment with hCDR1 resulted in the diminished expression of the pro-apoptotic factor JNK kinase along the p21Ras/MAP kinase pathway. Treatment with hCDR1 up-regulated $CD8^+$ regulatory cells as well. The latter cells were shown to be essential for the induction and the optimal suppressive function of $CD4^+CD25^+$ cells following treatment with hCDR1 (Figure 1).

The immunosuppressive cytokine, TGF-β, was shown to play a key role in the inhibition by hCDR1-induced $CD4^+CD25^+$ cells. This cytokine was demonstrated to be secreted by $CD4^+$ cells that were affected by hCDR1-induced immunoregulatory T cells. The up-regulated secretion of TGF-β as well as the down-regulation of activated autoreactive cells was associated with a decrease in the pathogenic cytokines (e.g. IFN-γ, IL-10). Treatment with hCDR1 down-regulated T cell adhesion and chemotaxis via the up-regulated TGF-β. The latter was associated with the down-regulation of ERK phosphorylation (known to participate in the signalling cascade that is involved in cell locomotion). Further, hCDR1 induced suppression of ERK-phosphorylation resulted in the diminished expression and function of a pair of key cell adhesion receptors, LFA-1 and CD44, which operate as accessory molecules in mediating APC-T-cell interactions. Finally, hCDR1 was shown to inhibit TCR signalling (ZAP-70 phosphorylation) and to up-regulate two negative regulators of TCR activation, namely Foxj1 and Foxo3a and two early growth response genes, namely Egr-2 and Egr-3 that are required for the induction of anergy (Figure 1). It is noteworthy that hCDR1 down-regulated in vitro the gene expression of pathogenic cytokines and apoptosis and up-regulated immunosuppressive factors and regulatory T cells in peripheral blood lymphocytes of lupus patients. Thus, treatment with hCDR1 leads to a cascade of events that culminates in the down-regulation of SLE-associated autoreactive responses and in the clinical improvement of SLE. hCDR1 is therefore a potential candidate for a novel specific treatment of SLE patients.

Selected publications

Ben-David H, Sela M and Mozes E. (2005) Down-regulation of myasthenogenic T cell responses by a dual altered peptide ligand via $CD4^+CD25^+$-regulated events leading to apoptosis. Proc. Natl. Acad. Sci. USA 102: 2028-2033.

Aruna BV, Sela M and Mozes E. (2005) Suppression of myasthenogenic responses of a T cell line by a dual altered peptide ligand by induction of $CD4^+CD25^+$ regulatory cells. Proc. Natl. Acad. Sci. USA 102: 10285-10290.

Aruna BV, Ben-David H, Sela M and Mozes E. (2006) A dual altered peptide ligand down-regulates myasthenogenic T cell responses and reverses experimental autoimmune myasthenia gravis via up-regulation of Fas-FasL-mediated apoptosis. Immunology 118: 413-424.

Aruna BV, Sela M and Mozes E. (2006) Down regulation of T cell responses to AChR and reversal of EAMG manifestations in mice by a dual altered peptide ligand via induction of CD4+CD25+ regulatory cells. J. Neuroimmunol. 177: 63-75.

Ben-David H, Aruna BV, Seger R, Sela M and Mozes E. (2006) A 50-kDa ERK-like protein is up-regulated by a dual altered peptide ligand that suppresses myasthenia gravis-associated responses. Proc. Natl. Acad. Sci. USA 103: 18232-18237.

Ben-David H, Aruna BV, Sela M and Mozes E. (2007) A dual altered peptide ligand inhibits myasthenia gravis associated responses by inducing phosphorylated extracellular-regulated kinase 1,2 that upregulates CD4+CD25+Foxp3+ cells. Scandinavian J. Immunol. 65:567-576.

Ben-David H, Sharabi A, Dayan M, Sela M and Mozes E. (2007) The role of CD8+CD28- regulatory cells in suppressing myasthenia gravis-associated responses by a dual altered peptide ligand. Proc. Natl. Acad. Sci. USA 104: 17459-17464.

Sela U, Hershkoviz R, Cahalon L, Lider O and Mozes E. (2005) Down-regulation of stromal cell-derived factor-1a-induced T cell chemotaxis by a peptide based on the complementarity-determining region 1 of an anti-DNA autoantibody via up-regulation of TGF-β secretion. J. Immunol. 174, 302-309.

Sela U, Mauermann N, Hershkoviz R, Zinger H, Dayan M, Cahalon L, Liu, JP Mozes E and Lider O. (2005) The inhibition of autoreactive T cell functions by a peptide based on the CDR1 of an anti-DNA autoantibody is via TGF-β mediated suppression of LFA-1 and CD44 expression and function. J. Immunol. 175, 7255-7263.

Rapoport MJ, Sharabi A, Aharoni D, Bloch O, Zinger H, Dayan M and Mozes E. (2005) Amelioration of SLE-like manifestations in (NZBxNZW) F1 mice following treatment with a peptide based on the complementarity determining region 1 of an autoantibody is associated with a down-regulation of apoptosis and of the pro-apoptotic factor JNK kinase. Clin. Immunol. 117, 262-270.

Sharabi A, Haviv A, Zinger H, Dayan M and Mozes E. (2006) Amelioration of murine lupus by a peptide, based on the complementarity determining region-1 of an autoantibody as compared to dexamethasone: Different effects on cytokines and apoptosis. Clin. Immunol. 119, 146-155.

Sela U, Dayan M, Hershkoviz R, Cahalon L, Lider O and Mozes E. (2006) The negative regulators Foxj1 and Foxo3a are up-regulated by a peptide that inhibits systemic lupus erythematosus-associated T cell responses. Eur. J. Immunol. 36, 2971-2980.

Sharabi A, Zinger H, Zborowsky

M, Sthoeger ZM and Mozes E. (2006) A peptide based on the complementarity-determining region 1 of an autoantibody ameliorates lupus by up-regulating $CD4^+CD25^+$ cells and TGF-β. Proc. Natl. Acad. Sci. USA 103, 8810-8815.

Elmann A, Sharabi A, Dayan M, Zinger H, Ophir R and Mozes E. (2007) Altered gene expression in mice with lupus treated with Edratide, a peptide that ameliorates the disease manifestations. Arthritis Rheum. 56, 2371-2381.

Sharabi A, Azulai H, Sthoeger ZM and Mozes E. (2007) Clinical amelioration of murine lupus by a peptide based on the complementarity determining region-1 of an autoantibody and by cyclophophamide: similarities and differences in the mechanisms of action. Immunology 121, 248-257.

Sharabi A, Luger D, Ben-David H, Dayan M, Zinger H and Mozes E. (2007) The role of apoptosis in the ameliorating effects of a CDR1-based peptide on lupus manifestations in a mouse model. J. Immunol. 179, 4979-4987.

Sela U, Dayan M, Hershkoviz R, Lider O, Mozes E. (2008) A Peptide that ameliorates lupus up-regulates the diminished expression of early growth response factors 2 and 3. J Immunol. 180, 1584-91.