

A Novel Synthetic Peptide for the Specific Treatment of Lupus: Clinical Effects and Mechanism of Action

Edna Mozes PhD, Uri Sela MD PhD and Amir Sharabi MD PhD

Department of Immunology, Weizmann Institute of Science, Rehovot, Israel

Key words: systemic lupus erythematosus, hCDR1, complementarity-determining region 1, CD4⁺CD25⁺ cells

IMAJ 2008;10:40–42

Systemic lupus erythematosus is an autoimmune disease that is characterized by the production of antibodies against nuclear antigens and by damage to multiple organs [1]. Several strains of mice that spontaneously develop a lupus-like disease were reported, of which the (NZBxNZW)F1 female mice are the most widely used [2]. In addition, we have previously established a model of experimentally induced SLE in various mouse strains [3,4].

As a potential candidate for the specific treatment of SLE patients, we have designed and synthesized a peptide that is based on the sequence of the complementarity-determining region 1 of a human monoclonal anti-DNA autoantibody [5]. The peptide designated hCDR1 was shown to mitigate the serological and clinical manifestations in both the spontaneous and induced models of SLE, to reduce the secretion and expression of the pathogenic cytokines interferon-gamma, interleukin-10, IL-1 β and tumor necrosis factor-alpha and to up-regulate the immunosuppressive cytokine transforming growth factor-beta [6]. It has been of utmost importance to elucidate the mechanisms underlying the beneficial effects of hCDR1.

hCDR1 ameliorates experimental SLE by up-regulating regulatory T cells

To further understand the mechanism by which hCDR1 ameliorates lupus manifestations we first tested whether the inhibitory capacity of hCDR1 could be transferred by immunocytes of hCDR1-treated mice. Indeed, adoptive transfer of cells from hCDR1-treated mice to (NZBxNZW)F1 female mice with full-blown SLE down-regulated all tested disease manifestations, suggesting the presence of a subpopulation of regulatory cells with suppressive activity [7]. The CD4⁺CD25⁺ regulatory T cells are the best characterized and are known to be protective against the development of autoimmune diseases [8]. We therefore studied the role of the latter cells in the beneficial effects of hCDR1. Treatment with hCDR1 resulted in an increase (30–40%) of CD4⁺CD25⁺ cells with regulatory characteristics such as CD45RB^{low}, CTLA-4 and Foxp3. Treatment with a control peptide or with the vehicle had no effect on either the magnitude or the inhibitory function

of the CD4⁺CD25⁺ cells. Depletion of CD25⁺ cells diminished significantly the therapeutic effects of hCDR1, whereas administration of an enriched CD4⁺CD25⁺ cell population was beneficial to the diseased mice. Mitigation of disease manifestations was associated with diminished secretion of the pathogenic cytokines (e.g., IFN γ and IL-10) and with up-regulation of TGF β whose secretion was shown to depend on the presence of hCDR1-induced CD4⁺CD25⁺ regulatory cells [7].

In addition to the CD4⁺ regulatory cells, CD8⁺ regulatory cells were reported to play an important role in autoimmune diseases including SLE [9]. In SLE patients the number of CD8⁺ cells is reduced, and the generation of suppressive CD8 cells is very limited [9]. We have previously shown that treatment of SLE-afflicted (NZBxNZW)F1 mice with hCDR1 increased the number of CD8⁺ cells, resulting in a significant decrease in the CD4:CD8 ratio [10]. We therefore investigated the possible role of CD8⁺ regulatory cells in the mechanism of action of hCDR1. We demonstrated that treatment of SLE-afflicted mice with hCDR1 up-regulated CD8⁺ cells, most of which belonged to the CD28⁻ compartment and expressed Foxp3. The *in vivo* depletion of the latter cells diminished the clinical improvement of the hCDR1-treated mice. More importantly, in the absence of CD8 T regulatory cells, CD4⁺CD25⁺ cells were unable to expand in the hCDR1-treated mice, and the expression of Foxp3 was reduced, thereby further interfering with the suppressive function of the CD4⁺CD25⁺ cells. To confirm the relative roles of CD8⁺CD28⁻ Foxp3⁺ and CD4⁺CD25⁺Foxp3⁺ cells in the mechanism of action of hCDR1, adoptive transfer experiments were performed with the hCDR1-induced regulatory cell populations. Administration of CD4⁺ regulatory cells into SLE-afflicted mice had more prominent beneficial effects on the serological and renal manifestations than that of adoptively transferred CD8⁺ regulatory cells, indicating the dominant role of CD4⁺CD25⁺Foxp3⁺ cells in the inhibitory function of hCDR1. Thus, CD8⁺CD28⁻ regulatory cells play a partial role in the inhibition of SLE-associated responses, but they are essential for the optimal expansion and function of the hCDR1-induced CD4⁺CD25⁺ regulatory cells, which play a key role in the suppression of SLE.

Treatment with hCDR1 down-regulates apoptosis in SLE-afflicted mice

Dysregulated apoptosis was reported to take place in SLE patients and in animal models of the disease [11]. We dem-

SLE = systemic lupus erythematosus

IL = interleukin

IFN γ = interferon-gamma

TGF β = transforming growth factor-beta

onstrated an increased rate of apoptosis in lymphocytes of (NZBxNZW)F1-afflicted mice. The latter was associated with elevated expression of caspase-8 and caspase-3 and with diminished expression of the anti-apoptotic molecule, Bcl-xL. Treatment with hCDRI down-regulated the caspases and up-regulated Bcl-xL in association with reduced rates of apoptosis and amelioration of SLE manifestations. Co-incubation of Bcl-xL inhibitors with hCDRI-treated cells abrogated the ability of hCDRI to reduce the secretion of the pathogenic cytokines. Further, a markedly higher expression of Bcl-xL in CD4⁺CD25⁺Foxp3⁺ regulatory cells of hCDRI-treated mice than in CD4⁺CD25⁺ cells of vehicle-treated mice was demonstrated. Moreover, the Bcl-xL-expressing regulatory cells induced the expression of Bcl-xL in CD4⁺CD25⁻ cells of the SLE-afflicted mice. Thus, the reduction of apoptosis and the up-regulation of Bcl-xL contribute to at least part of the beneficial effects of hCDRI on SLE manifestations [12].

hCDRI affects T cell receptor signaling and regulation

Immunization of mice with the monoclonal anti-DNA antibody designated 16/6 idiotype resulted in the induction of experimental SLE [3]. Further, administration of hCDRI concomitantly with the immunization with the 16/6 Id resulted, already 10 days following immunization, in diminished specific T cell responses including chemotaxis, adhesion and proliferation in association with the inhibition of ERK phosphorylation [13]. Injection of hCDRI concomitant with the immunization with hCDRI diminished significantly the secretion and expression of INF γ in T cells of the treated mice. The latter was associated with a lower expression of T-bet, which is a master gene that plays an important role in coordinating Th1 differentiation and induction of INF γ expression [14]. Treatment with hCDRI inhibited also the activation of nuclear factor-kappa B, another Th1-associated factor that has a pivotal role in the specific regulation of Th1 development [15]. To determine whether the inhibition of NF- κ B activation and T-bet expression by hCDRI is associated with its effect on the upstream T cell receptor signaling, we studied the phosphorylation of ZAP-70 and demonstrated its marked inhibition by hCDRI as compared to T cells derived from non-treated 16/6 id immunized mice [16].

Foxj1 and Foxo3a are two members of the forkhead family that were shown to be actively involved in the negative regulation of T cells by maintaining NF- κ B in an inactive state. Both factors were shown to be diminished in lupus-prone mice [17,18]. *In vivo* treatment with hCDRI up-regulated significantly these two transcription factors, both 10 days after immunization with 16/6 Id and in (NZBxNZW)F1 mice with full-blown SLE. The addition of TGF β , which was elevated following treatment with hCDRI, to T cells from 16/6 Id-immunized mice up-regulated Foxj1 and Foxo3a mRNA expression, similar to the *in vivo* treatment with hCDRI. In contrast, anti-TGF β antibodies that were added to hCDRI-treated T cells abrogated its effect [16]. Thus, TGF β that is

elevated by hCDRI contributes to the up-regulation of Foxj1 and Foxo3a and to the subsequent events that lead to the diminished INF γ production.

A pair of transcription factors, early growth response 2 (Egr-2) and 3 (Egr-3), are negative regulators of T cell activation that were shown to be important in anergy [19]. Because autoreactive T cells in lupus were reported to be resistant to anergy [20], it was of interest to find out whether hCDRI affects anergy and if the effects of hCDRI are mediated by the two transcription factors, Egr-2 and Egr-3. We showed that treatment with hCDRI induced anergy in autoreactive T cells, as manifested by the diminished IL-2 mRNA expression and proliferation. The latter was reversible upon addition of rIL-2. Further, treatment with hCDRI significantly up-regulated Egr-2 and Egr-3 – both soon after immunization with 16/6 Id and in SLE-afflicted (NZBxNZW)F1 mice. The increase in Egr-2 and Egr-3 expression was associated with down-regulation of Akt phosphorylation and up-regulation of TGF β . Inhibition of Akt in T cells of hCDRI-treated mice decreased, whereas silencing of Egr-2 and Egr-3 in T cells of hCDRI-treated mice increased the secretion of INF γ . Thus, the down-regulation of Akt phosphorylation by hCDRI leads to the up-regulated expression of Egr-2 and Egr-3, resulting in the inhibition of the pathogenic cytokine INF γ and in the induction of anergy.

Conclusions

Treatment with hCDRI up-regulates Foxp3 expressing CD4⁺CD25⁺ as well as CD8⁺CD28⁻ regulatory cells. The latter cells are required for the induction and optimal inhibitory function of the CD4⁺CD25⁺ cells. Reduction of apoptosis by hCDRI contributes to at least part of the beneficial effects of hCDRI. Finally, hCDRI inhibits T cell receptor signaling and up-regulates the negative regulators of T cell receptor activation, Foxj1 and Foxo3a and Egr-2 and Egr-3, which are required for anergy induction. Thus, treatment with hCDRI leads to a cascade of events that culminate in the clinical improvement of SLE.

Acknowledgments. Supported by TEVA Pharmaceutical Industries, Israel.

References

1. Hahn BH. An overview of the pathogenesis of systemic lupus erythematosus. In: Wallace D, Hahn BH, eds. *Dubios' Lupus Erythematosus*. Philadelphia: Lippincott Williams & Wilkins, 2002: 87–92.
2. Theofilopoulos AN. Murine models of lupus. In: Lahita RG, ed. *Systemic Lupus Erythematosus*. New York: Churchill Livingstone, 1992:121–94.
3. Mendlovic S, Brocke S, Shoenfeld Y, et al. Induction of a systemic lupus erythematosus-like disease in mice by a common human anti-DNA idiotype. *Proc Natl Acad Sci USA* 1988;85:2260–4.
4. Mendlovic S, Brocke S, Fricke H, Shoenfeld Y, Bakimer R, Mozes E. The genetic regulation of the induction of experimental SLE. *Immunology* 1990;69:228–36.
5. Waisman A, Shoenfeld Y, Blank M, Ruiz PJ, Mozes E. The pathogenic human monoclonal anti-DNA that induces experimental

Erg = early growth response

NF- κ B = nuclear factor-kappa B

- systemic lupus erythematosus in mice is encoded by a VH4 gene segment. *Int Immunol* 1995;7:689–96.
- Luger D, Dayan M, Zinger H, Liu J-P, Mozes E. A peptide based on the complementarity determining region I of a human monoclonal autoantibody ameliorates spontaneous and induced lupus manifestations in correlation with cytokine immunomodulation. *J Clin Immunol* 2004;24:579–90.
 - Sharabi A, Zinger H, Zborowsky M, Stoeber ZM, Mozes E. A peptide based on the complementarity-determining region I of an autoantibody ameliorates lupus by up-regulating CD4⁺CD25⁺ cells and TGF- β . *Proc Natl Acad Sci USA* 2006;103:8810–15.
 - Shevach EM. Regulatory T cells in autoimmunity. *Annu Rev Immunol* 2000;18:423–49.
 - Filaci G, Bacilieri S, Fravega M, et al. Impairment of CD8⁺ T suppressor cell function in patients with active systemic lupus erythematosus. *J Immunol* 2001;166:6452–7.
 - Sharabi A, Azulai H, Stoeber MZ, Mozes E. Clinical amelioration of murine lupus by a peptide based on the complementarity determining region-I of an autoantibody and by cyclophosphamide: similarities and differences in the mechanisms of action. *Immunology* 2007;121:248–57.
 - Kaplan MJ. Apoptosis in systemic lupus erythematosus. *Clin Immunol* 2004;112:210–18.
 - Sharabi A, Luger D, Ben-David H, Dayan M, Zinger H, Mozes E. The role of apoptosis in the ameliorating effects of a CDR1-based peptide on lupus manifestations in a mouse model. *J Immunol* 2007;179:4979–87.
 - Sela U, Hershkoviz R, Cahalon L, Lider O, Mozes E. Down-regulation of stromal cell-derived factor-1 α -induced T cell chemotaxis by a peptide based on the complementarity-determining region I of an anti-DNA autoantibody via up-regulation of TGF- β secretion. *J Immunol* 2005;174:302–9.
 - Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, direct Th1 lineage commitment. *Cell* 2000;100:655–69.
 - Murphy KM, Ouyang W, Farrar JD, et al. Signaling and transcription in T helper development. *Annu Rev Immunol* 2000;18:451–94.
 - Sela U, Dayan M, Hershkoviz R, Cahalon L, Lider O, Mozes E. The negative regulators Foxj1 and Foxo3a are up-regulated by a peptide that inhibits systemic lupus erythematosus-associated T cell responses. *Eur J Immunol* 2006;36:2971–80.
 - Lin L, Spoor MS, Gerth AJ, Brody SL, Peng SL. Modulation of Th1 activation by the NF-kappaB repressor Foxj1. *Science* 2004;303:1017–20.
 - Lin L, Horn JH, Peng SL. Regulation of NF-kappaB, Th activation, and autoinflammation by forkhead transcription factor Foxo3a. *Immunity* 2004;21:203–13.
 - Safford M, Collins S, Lutz MA, et al. Egr-2 and Egr-3 are negative regulators of T cell activation. *Nat Immunol* 2005;6:472–80.
 - Xu L, Zhang L, Yi Y, Kang HK, Datta SK. Human lupus T cells resist inactivation and escape death by upregulating COX-2. *Nat Med* 2004;10:411–15.

Correspondence: Dr. E. Mozes, Dept. of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel.

Phone: (972-8) 934-3646

Fax: (972-8) 934-4173

email: edna.mozes@weizmann.ac.il