The tolerogenic peptide hCDR1 downregulates pathogenic cytokines and apoptosis and upregulates immunosuppressive molecules and regulatory T cells in peripheral blood mononuclear cells of lupus patients

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ABSTRACT

A tolerogenic peptide, hCDR1, ameliorated murine lupus via the upregulation of functional regulatory cells and by immunomodulating cytokine production. In the present study we analyzed the ability of hCDR1 to similarly affect gene expression and regulatory T cells when incubated with peripheral blood mononuclear cells (PBMC) of lupus patients. To this end, peripheral blood mononuclear cells (PBMC) of 11 lupus patients and five gender- and age-matched healthy controls were cultured with hCDR1 or a control peptide. Gene expression and regulatory T-cells were assessed. hCDR1 significantly downregulated interleukin (IL)–1β, interferon (IFN)–γ, and IL-10 gene expression. Furthermore, hCDR1 upregulated the expression of the anti-apoptotic Bcl-xL molecule and downregulated the pro-apoptotic caspase-3, resulting in reduced rates of apoptosis. hCDR1 increased the expression of transforming growth factor (TGF)–β, Foxp3 and the negative regulators Foxj1 and Foxo3a. No significant effects were observed using a control peptide or when PBMC of healthy donors were incubated with hCDR1. The elevated gene expression of Foxp3 was due to hCDR1-induced upregulation of TGF-β, resulting in an increase of CD4+CD25+Foxp3+ functional, regulatory cells. The ability of the regulatory cells to diminish IFN-γ expression and to upregulate TGF–β was abrogated after the addition of a neutralizing anti-CD25 antibody, confirming their role in the beneficial effects of hCDR1.

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1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies and impaired function of B and T cells accompanied by systemic clinical manifestations [1,2]. Previous studies demonstrated the role of cytokines [3,4], apoptosis [5,6] and dysfunction of regulatory T-cells [7,8] in the pathogenesis of murine and human SLE. Our laboratory designed a peptide, designated hCDR1 [9], that is based on the sequence of the complementarity-determining region (CDR) 1 of a human anti-DNA monoclonal antibody (mAb) that bears a major idiotype (Id), namely 16/6Id [10]. hCDR1 was shown to ameliorate serologic and clinical manifestations of murine lupus that was either induced in BALB/c mice or spontaneously developed in (NZBxNZW)F1 mice [11,12]. Attempts to elucidate the mechanisms of action of hCDR1 indicated that the peptide, shown to bind to major histocompatibility complex II on antigen-presenting cells and to inhibit T-cell receptor signaling [13], downregulated the pathogenic cytokines interleukin (IL)–1β, interferon (IFN)–γ, and IL-10 [14] and upregulated the immunosuppressive cytokine transforming growth factor (TGF)–β [15]. Furthermore, treatment of mice with induced or spontaneous SLE with hCDR1 resulted in reduced apoptosis rates of T cells [16–18]. In addition, hCDR1 induced specifically CD4+CD25+Foxp3+ regulatory cells [15,19] and upregulated the negative T-cell regulators Foxj1 and Foxo3a [13]. hCDR1 was shown to affect dendritic cells as well by reducing their number and inducing an immature phenotype in the remaining dendritic cells [20]. Thus, the amelioration of lupus by hCDR1 is a result of its effects on different cell populations and signaling pathways.

We have previously shown that hCDR1 downregulated, in vitro, the proliferation of PBMC of SLE patients in association with an increased production of TGF–β [9]. Moreover, we were able to establish a human model of SLE by transferring PBMC of lupus patients into severe combined immunodeficient (SCID) mice [21]. Treatment with hCDR1 ameliorated serologic (human anti-DNA antibodies) and clinical lupus-related manifestations in the latter SCID model of human SLE [22]. In view of the various immunomodulatory effects of hCDR1 in the murine models of lupus, it was of interest to determine the effects of the peptide on PBMC obtained from SLE patients. To this end, we studied the in vitro effects of hCDR1 incubated with PBMC of SLE patients on gene expression of the pathogenic cytokines (IL-1β, IFN–γ, and IL-10) and of the pro-apoptotic caspase3 and the anti-apoptotic Bcl-xL molecules. In addition, we assessed the effects of hCDR1 on gene expression of...
TGF-β, FoxP3, Foxj1, and Foxxo3a and on the number and function of CD4+CD25+FoxP3+ regulatory cells.

2. Subjects and methods

2.1. Patients

Eleven lupus patients (nine female and two male) and five sex- and age-matched healthy controls participated in our study. All patients were diagnosed according to four or more American College of Rheumatology (ACR) SLE diagnostic criteria [23]. The mean ± SD age of the patients was 49.09 ± 13.20 years (range, 27–62 years). Their mean ± SD disease activity score, defined by SLE disease activity index (SLEDAI) [24], was 6.55 ± 6.30 (range, 2–22). The main current clinical manifestations of the patients were arthritis (64%), mucocutaneous (54%), renal (27%), and pleuritis/pericarditis (18%). Six patients were treated with corticosteroids at the time of the study (daily dose of 11.0 ± 23.4 mg prednisone; range, 2.5–80.0 mg). Seven patients were treated with Plaquanil (400 mg/day) on a regular basis. All participants signed an informed consent form before the initiation of the study, which was approved by the Ethic Committee of Kaplan Medical Center.

2.2. Synthetic peptides

A peptide, GYYWSWIRQQPGKEEGWIG, designated as hCDR1 [9], was synthesized by Polypeptide Laboratories (Los Angeles, CA). A control peptide, SKGIPQYGWPWEGRYELI, contains the same amino acids of hCDR1 in a scrambled order. The control peptide binds MHC class II with an avidity similar to that of hCDR1.

2.3. Cultures

PBMC were isolated from heparinized venous blood by Ficoll-Hypaque (Pharmacia) density gradient centrifugation [25]. PBMC (5 × 10^6/ml) were cultured in enriched RPMI-1640 medium containing 10% fetal calf serum [9] for 24 hours in 7.5% CO2 at 37°C in the presence of medium, hCDR1 or the control peptide (both at 25 μg/ml).

2.4. In vitro assays

PBMC (5 × 10^6/ml) of individual patients that were previously shown to respond to 16/6 Id stimulation, were cultured in enriched medium for 24 hours with either hCDR1 alone (25 μg/ml) or hCDR1 and anti-CD25 (10 μg/ml) mAb (daclizumab; Zenapax; Roche Pharmaceuticals, Basel, Switzerland). The cells of the latter cultures were washed and incubated in enriched medium with untreated PBMC (ratio 1:3) of the matched patients in the presence of 16/6 Id (10 μg/ml) for 36 hours. Thereafter, RNA was extracted and gene expression was determined by real time reverse transcription-polymerase chain reaction (RT-PCR).

2.5. 16/6 Id bearing antibodies

16/6 Id-bearing antibodies in the sera of SLE patients and healthy controls were determined by enzyme-linked immunoabsorbent assay (ELISA). Briefly, 96-well plates (Nunc, Denmark) were incubated with 50 μl of rabbit anti-16/6 Id (IgG fraction) antibody or control Rabbit IgG (0.5 μg/ml). After blocking, sera (dilution 1:10–1:1000) were added. After incubation with goat anti-human IgG (γ-chain specific) [Jackson, West Grove, PA] ABTS (2,2 Azino-bis [3-Ethylbenz-Thiazoline-6 Sulfonic acid; Sigma, Israel)] was added and optical density (OD) was read at 405 nm. Nonspecific binding (to the control rabbit IgG) was subtracted. The mean OD ± 2SD of 50 control sera was defined as upper limit of normal binding. Only one control serum (2%) was determined to be positive.

2.6. Real-time RT-PCR

Expression of mRNA was determined by real-time RT-PCR using LightCycler (Roche Mannheim, Germany). Total RNA was isolated from cultures of PBMC. The RNA was then reverse transcribed to prepare complementary DNA (cDNA) using Maloney murine leukemia virus reverse transcriptase (Promega, Madison, WI). The resulting cDNA was subjected to real-time RT-PCR according to the manufacturer’s instructions. The following primer sequences (forward and reverse, respectively) were used: IL-1β (5’-cagaaaacatgccgct-3’, 5’-gccatcctttagggcag-3’), IFN-γ (5’-tccagttgcaaggaac-3’, 5’-ggcagatacggatatgcac-3’), IL-10 (5’-aaacatggagttgcttgcacctc-3’, 5’-acctaggctggcttaccc-3’), caspase-3 (5’-cagagagccctctactc-3’, 5’-ggtctagctgccctca-3’), Bel-1 (5’-agcatgctacagacgc-3’, 5’-aagcaaattcaaggggcac-3’), TGF-β (5’-cgaagactcggctcagggc-3’, 5’-actttgtcatagttgcgttg-3’), FoxP3 (5’-ccaaacatggcactt-3’, 5’-ctgcttcttcgggacact-3’), Foxj1 (5’-acggcacttctgctc-3’, 5’-gctctgtgtgccttttg-3’), Foxo3a (5’-ctctttcgcctctc-3’, 5’-gaatttgggccgca-3’) and GAPDH (5’-cctggacactgctcag-3’, 5’-gctggagggcacatgca-3’). The levels of GAPDH were used to normalize all other genes. Results are expressed as percent relative expression.

2.7. Flow cytometry

The following antibodies were used in the study: anti-human CD4 PE (RPA-T4), and its isotype control (BD Pharmingen, San Diego, CA); anti-human CD25 APC (4E3) and its isotype control (Milenyi Biotec Inc. Auburn, CA); and anti-human FoxP3 FITC (236A/E7) (eBioscience, San Diego, CA). Cells (1 × 10^6/sample) were washed with staining buffer (PBS, 0.5% BSA, 2 mmol/l EDTA) and

![Fig. 1. hCDR downregulates IL-1β, IFN-γ, and IL-10 gene expression. PBMC of SLE patients and healthy controls were cultured (5 × 10^6 cells/well) for 24 hours in the presence of medium, hCDR1, or the scrambled control peptide (25 μg/ml). Gene expression was determined by real-time RT-PCR. Results are presented as the mean ± SE percentage of gene expression compared with cultures with medium (considered as 100%).]
stained for 20 minutes at 4°C with the relevant mAb. After surface staining, cells were fixed and permeabilized for intracellular staining of FoxP3 according to the manufacturer’s protocol.

2.8. Statistical analyses

The nonparametric Mann-Whitney and unpaired Student’s t tests were used for statistical analyses. Values of $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Effects of hCDR1 on cytokine gene expression

Amelioration of murine lupus following treatment with hCDR1 was associated with downregulation of pathogenic cytokines [14]. To ascertain whether hCDR1 could similarly regulate cytokine production in SLE patients, PBMC of 11 lupus patients and five healthy controls were cultured for 24 hours with medium, hCDR1, or the scrambled control peptide. Preliminary experiments were performed to determine the optimal concentration of hCDR1 to be used in the culture. Thus, PBMC of four patients were cultured in the presence of two concentrations of hCDR1, namely, 50 and 25 μg/ml. Thereafter RNA was extracted and gene expression was determined by real-time RT-PCR. The results for all four patients indicated that the addition of 25 μg/ml hCDR1 to the PBMC in the culture resulted in a more prominent immunomodulation of gene expression than the higher concentration of 50 μg/ml. Therefore, 25 μg/ml of hCDR1 were used in all further experiments. Fig. 1 shows that in vitro incubation of PBMC of SLE patients with hCDR1, significantly reduced gene expression of IL-1β, IFN-γ, and IL-10 compared with PBMC of the same patients cultured with medium or with the control peptide. It should be noted that although the control peptide upregulated the expression of IL-1β and IL-10, the effects were not significant ($p = 0.64$ and 0.85, respectively) when compared with PBMC incubated in the presence of medium. The effects of hCDR1 on the expression of the same genes in PBMC of healthy controls are also shown in Fig. 1. It can be seen that the mean percent expression of the three pathogenic cytokines was insignificantly upregulated after incubation of PBMC of healthy donors in the presence of hCDR1.

3.2. Effects of hCDR1 on apoptosis

Because the beneficial effects of hCDR1 on murine lupus correlated with a decreased rate of apoptosis [16–19], we studied whether hCDR1 affects, in vitro, the expression of the pro-apoptotic (caspase-3) and the anti-apoptotic (Bcl-xL) molecules in PBMC of SLE patients. As shown in Fig. 2, hCDR1, but not the control peptide, significantly decreased caspase-3 (53% and 89% compared with cultures with medium or control peptide, respectively) and increased Bcl-xL (38%, $p = 0.055$ and 70%, $p = 0.001$, compared with cultures with medium or control peptide, respectively) gene expression. The effects on the expression of both genes in PBMC of healthy controls were unremarkable as demonstrated in Fig. 2. Furthermore, as can be seen in Fig. 2B, incubation of PBMC of SLE patients in the presence of hCDR1 reduced the rates of apoptosis as determined by cells stained positively to Annexin V and negatively to propidium iodide.

3.3. Effects of hCDR1 on gene expression of Foxj1 and Foxo3a

Foxj1 and Foxo3a are transcription factors that play a role in negative regulation of Th1 responses [26]. Treatment of SLE af-

![Fig. 2. hCDR1 downregulates caspase-3 and upregulates Bcl-xL gene expression. PBMC of SLE patients and healthy controls were cultured (5 × 10⁶ cells/well) for 24 hours in the presence of medium, hCDR1, or the scrambled control peptide (25 μg/ml). (A) Gene expression was determined by real-time RT-PCR. Results are presented as the mean ± SE percentage of gene expression compared with cultures with medium (considered as 100%). (B) Representative dot plots depicting double staining of the cells with Annexin V and propidium iodide as well as the mean (±SE) percentage of apoptotic cells (those stained positively to Annexin V and negatively to propidium iodide) compared with cultures with medium (considered as 100%).](image1)

![Fig. 3. hCDR1 upregulates Foxj1 and Foxo3a gene expression. PBMC of SLE patients and healthy controls were cultured (5 × 10⁶ cells/well) for 24 hours in the presence of medium, hCDR1, or the scrambled control peptide (25 μg/ml). Gene expression was determined by real-time RT-PCR. Results are presented as the mean ± SE percentage of gene expression compared with cultures with medium (considered as 100%).](image2)
timated models after treatment with hCDR1 [15,18,19]. We therefore assessed the effects of hCDR1 on TGF-β and FoxP3 gene expression. Fig. 4 demonstrates a significant upregulation in the expression of TGF-β (375%) and of FoxP3 (228%) when compared with controls with medium. In contrast, the control peptide decreased gene expression of both, TGF-β, and FoxP3 in PBMC of the same SLE patients (Fig. 4). Although insignificantly, hCDR1 also increased gene expression of TGF-β and FoxP3 in PBMC of healthy controls, as evident in Fig. 4.

3.5. Upregulation of functional CD4+CD25+FoxP3+ cells by hCDR1

The regulatory master gene FoxP3 is mainly expressed in regulatory T cells [7,8]. To determine whether the hCDR1-induced up-regulation of FoxP3 gene expression was caused by an increase in CD4+CD25+ cells, PBMC of four SLE patients and three healthy controls were incubated in the presence or absence of hCDR1 before staining with CD4, CD25, and FoxP3. Representative results of three independent experiments are shown in Fig. 5. As shown, the frequency of CD4+CD25+ (3.4% vs 5.2%) and of CD4+CD25+ FoxP3+ regulatory cells (1.4% vs 3.5%) in PBMC of SLE patients incubated in medium were lower compared with cultures of healthy controls. Furthermore, the mean fluorescence intensity (MFI) of FoxP3 in the CD4+CD25+ cells of SLE patients was also lower compared with the healthy controls (MFI, 43 vs 54). Incubation of PBMC of SLE patients with hCDR1 increased the frequency of CD4+CD25+ (5.5% vs 3.4%) and of CD4+CD25+ FoxP3+ cells (2.5% vs 1.4%). The effect of hCDR1 on PBMC of healthy controls was less prominent (5.7% vs 5.2% and 4.1% vs 3.5% for CD4+CD25+ and CD4+CD25+ FoxP3+ cells, respectively). Furthermore, the hCDR1-induced increase in MFI of FoxP3 in CD4+CD25+ gated cells was observed only in PBMC of SLE patients.

To determine the role of TGF-β in mediating the expression of FoxP3, we incubated PBMC of SLE patients in the presence of hCDR1, with or without anti–TGF-β-neutralizing antibody. Results presented in Fig. 6 show that the upregulated expression of FoxP3 in the CD4+CD25+ cells incubated with hCDR1 was diminished after the addition of TGF-β–specific neutralizing antibody. In agreement, it is also evident in Fig. 6 that the addition of rhTGF-β to the PBMC of SLE patients upregulated FoxP3 expression to levels comparable to those of hCDR1.

To confirm that the hCDR1-induced CD4+CD25+FoxP3+ cells are responsible for the suppressive effects observed after incubation of PBMC with hCDR1, PBMC of SLE patients were incubated for 24 hours in the presence of hCDR1 (25 μg/ml) or in the presence of hCDR1 and a neutralizing anti-CD25 mAb (10 μg/ml).

**Fig. 4.** hCDR1 upregulates TGF-β and FoxP3 gene expression. PBMC of SLE patients and healthy controls were cultured (5 × 10^6 cells/well) for 24 hours in the presence of medium, hCDR1, or the scrambled control peptide (25 μg/ml). Gene expression was determined by real-time RT-PCR. Results are presented as the mean ± SE percentage of gene expression compared with cultures with medium (considered as 100%).

**Fig. 5.** hCDR1 upregulates FoxP3-expressing CD4+CD25+ cells. PBMC of SLE patients and healthy controls were cultured (5 × 10^6 cells/well) for 24 hours in the presence of medium or hCDR1 (25 μg/ml). Thereafter, cells were stained for the expression of CD4, CD25, and FoxP3. Representative results of three independent experiments are shown. Percentages in dot plots indicate the rate of CD4+CD25+ cells. Histograms show staining with FoxP3 (solid line) or with isotype control (dashed line) in CD4+CD25+ gated cells. Numbers in histograms indicate the percentages of CD4+CD25+ cells that express FoxP3 (top), and the MFI of FoxP3 expression in CD4+CD25+ gated cells (bottom).
The cells were then collected and cultured with PBMC of the same lupus patients in a ratio of 1:3 (cells incubated with either hCDR1 or hCDR1 and a neutralizing anti-CD25 mAb: untreated PBMC), in the presence of 16/6Id (10 μg/ml) as schematically shown in Fig. 7A. After 36 hours of incubation, mRNA was prepared of the cultured cells and the expression of the inflammatory cytokine IFN-γ and of the immunosuppressive cytokine TGF-β were determined by real time RT-PCR. Results shown in Fig. 7B indicate that the inhibitory effect on IFN-γ expression of the hCDR1-induced cells was abrogated when the regulatory cells were incubated in the presence of the anti-CD25 mAb. Furthermore, incubation of hCDR1 induced cells with the anti-CD25 neutralizing mAb downregulated the expression of the TGF-β gene that was elevated after incubation of PBMC of lupus patients with hCDR1-induced regulatory cells.

3.6. Lack of correlation between the effects of hCDR1 and the presence of 16/6Id-bearing antibodies

To determine whether the immunomodulatory effects of hCDR1 on PBMC of SLE patients were related to the presence of 16/6Id-bearing antibodies, we measured the expression of 16/6Id in sera of our SLE patients and healthy controls. Four of 11 (36%) of the SLE patients and none of the five healthy controls possessed such antibodies. Thus it appears that hCDR1 affects PBMC in all SLE patients, regardless of their 16/6Id expression.

4. Discussion

The main findings of our study are that hCDR1 is capable of immunomodulating in vitro cytokines, apoptosis, and immunosuppressive molecules in PBMC of SLE patients. Moreover, hCDR1 up-regulates FoxP3 expression and CD4<sup>+</sup>CD25<sup>+</sup> functional regulatory cells.

Cytokines play an important role in the pathogenesis of human [3] and murine [4] SLE. High levels of IFN-γ, IL-1β, and IL-10 were reported in murine models of lupus [4,14] and in SLE patients [3,27]. Immunosuppressive treatment of SLE patients [28] and of (NZBxNZW)F1 mice [17,19] as well as hCDR1 treatment of murine lupus [14] downregulated those cytokines. Similarly to its in vivo effects in mice, hCDR1 significantly decreased, in the present study, gene expression of IL-1β, IFN-γ and IL-10 in PBMC of SLE patients (Fig. 1).

Apoptosis is involved in the pathogenesis of murine and human SLE [5,6]. PBMC of active lupus patients were shown to exhibit an increased rate of apoptosis [6,29], and administration of apoptotic lymphocytes to (NZBxNZW)F1 mice led to accelerated lupus-related manifestations [5]. Moreover, an increased rate of apoptosis with an upregulation of the pro-apoptotic caspase-3 and caspase-8 and downregulation of the anti-apoptotic Bcl-xL was demonstrated in (NZBxNZW)F1 lupus–prone mice and in mice with induced SLE [17,18]. The ameliorating effects of treatment with hCDR1 were due, at least in part, to the downregulated apoptosis that correlated with diminished caspase-3 and elevated Bcl-xL[18].

Fig. 6. TGF-β mediates the hCDR1-induced expression of FoxP3 in CD4<sup>+</sup>CD25<sup>+</sup> cells of SLE-derived PBMC. The PBMC of SLE patients were cultured (5 × 10<sup>6</sup> cells/well) for 24 hours in the presence of medium, rhTGF-β (250 pg/ml), hCDR1 (25 μg/ml) alone or together with an anti-TGF-β neutralizing antibody (10 μg/ml). Thereafter, cells were stained for the expression of CD4, CD25, and FoxP3, and the mean fluorescence intensity (MFI) of FoxP3 was determined in gated CD4<sup>+</sup>CD25<sup>+</sup> cells. Results are presented as the mean ± SE percentage of MFI.

Fig. 7. hCDR1-induced CD4<sup>+</sup>CD25<sup>+</sup> cells are responsible for suppressing IFN-γ and upregulating TGF-β mRNA expression of SLE-derived PBMC. (A) Illustration of the experimental procedure. PBMC of individual SLE patients were cultured (5 × 10<sup>6</sup>/ml) in enriched medium for 24 hours in the presence of hCDR1 (25 μg/ml), together or without anti-CD25 (10 μg/ml) mAb. The cultured cells were washed and reincubated with untreated PBMC (ratio 1:3) of the matched patients in the presence of 16/6Id (10 μg/ml) for 36 hours. (B) Gene expression of IFN-γ and TGF-β was determined in the RNA extracts of the cell cultures.
In agreement, hCDR1 downregulated caspase-3, upregulated Bcl-xL, gene expression, and reduced the rates of apoptosis in cultures of PBMC of lupus patients (Fig. 2).

TGF-β plays a pivotal role in suppressing autoimmune disorders [26,30]. Thus, low levels of TGF-β were determined in active SLE patients [31], whereas B-cell deletion using rituximab increased TGF-β production [32]. Furthermore, TGF-β knockout mice developed lethal lupus-like disease [33], and the ameliorative effects of hCDR1 in SLE-afflicted mice were associated with upregulated TGF-β mainly in CD4 effector cells [15]. Similarly, as demonstrated here, hCDR1 upregulated TGF-β in PBMC of SLE patients (Fig. 4).

CD4+CD25+FoxP3+ regulatory cells play a major role in maintaining immunologic homeostasis [7,8]. Indeed, low levels of CD4+CD25+FoxP3+ cells and reduced immunosuppressive functions in PBMC of active lupus patients were reported [34,35]. Adaptive transfer of hCDR1-induced CD4+CD25+FoxP3+ regulatory cells led to amelioration of the disease manifestations in lupus-affected mice [15,19]. Here we were able to demonstrate an upregulation of FoxP3 gene expression (Fig. 4) and an increased frequency of CD4+CD25+FoxP3+ cells in cultures of PBMC of SLE patients after incubation with hCDR1 (Fig. 5). It was recently reported that in vitro stimulation of T cells of SLE patients upregulated FoxP3 expression with a parallel increase in suppressive functions [36]. In contrast to the generation of nonspecific (anti-CD3 and IL-2 stimulation) regulatory T-cells in the latter study, hCDR1 specifically upregulated FoxP3+ regulatory cells since the control peptide did not have such an effect (Fig. 4). It appears that hCDR1 upregulated the frequency of CD4+CD25+ cells as well as the expression and intensity of FoxP3 (Fig. 5). The upregulated FoxP3 was due, at least partially, to the hCDR1-mediated upregulation of TGF-β expression (Fig. 6). Furthermore, the hCDR1 in vitro induced cells were functional because they efficiently suppressed the expression of IFN-γ and upregulated TGF-β when incubated with untreated PBMC of the SLE patients. The immunomodulating activity of the hCDR1 induced cells was abrogated by a neutralizing anti-CD25 mAb (Fig. 7), thereby indicating that the hCDR1 induced CD4+CD25+FoxP3+ cells played a key role in the regulation of SLE associated genes. Our findings are supported by a recent report of an anti-DNA Ig–derived peptide that upregulated in vitro the number and function of CD4+CD25+ cells of SLE patients [37].

FoxJ1 and FoxO3a were shown to be involved in negative regulation of T-cell activation [26,38,39]. Indeed, FoxJ1 and FoxO3a knockout mice demonstrated systemic autoimmune disorders [40], and polymorphism of FoxJ1 gene correlated with the presence of human SLE [41]. Treatment with hCDR1 upregulated FoxJ1 in murine models of SLE [13]. Similarly, we demonstrate here that hCDR1 significantly increased the expression of both negative regulators in PBMC of SLE patients (Fig. 3).

The effects of hCDR1 on PBMC of healthy controls were statistically insignificant (Figs. 1–4). Nevertheless, hCDR1 upregulated, although to a lesser extent and insignificantly, gene expression of inhibitory factors (Figs. 3 and 4) and the frequency of FoxP3 regulatory cells (Fig. 5) in healthy controls. These observations are in agreement with our previous report demonstrating that hCDR1 induced specific CD4+CD25+Foxp3+ regulatory cells in healthy mice as well [15].

Although hCDR1 is based on an anti-DNA mAb that bears the 16/6id [9,10] its in vitro immunomodulatory effects on PBMC of SLE patients were independent of the presence of 16/6id-bearing antibodies. Whereas PBMC of all lupus patients studied were affected by the peptide, 16/6id-bearing antibodies were present in only four of them. In accordance, our previous studies demonstrated that hCDR1 ameliorated lupus in (NZBxNZW)F1 mice that do not produce 16/6id bearing anti-DNA antibodies [14]. It is therefore suggested that hCDR1 is capable of affecting a broad population of lupus patients.

The present study clearly demonstrated that hCDR1 immunomodulated gene expression of cytokines, apoptosis, and inhibitory factors in PBMC of SLE patients. Based on our previous studies in murine models of SLE, it is likely that the observed in vitro hCDR1 effects on one immunologic pathway may cause a consequent effect on another. Thus, downregulation of IFN-γ and IL-10 may contribute to the reduced expression of pro-apoptotic genes [42,43], whereas induced expression of the anti-apoptotic molecule Bcl-xL can lead to and sustain low levels of those cytokines [18]. Furthermore, the upregulated TGF-β might induce the expression of the regulatory master gene FoxP3 [44] along with the FoxJ1 and FoxO3a, negative regulators that may decrease IFN-γ gene expression [13]. It is likely that hCDR1 ameliorates PBMC of SLE patients via similar mechanisms observed in murine lupus models, suggesting that hCDR1 is a novel therapeutic mean for SLE patients.

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