Amelioration of Brain Pathology and Behavioral Dysfunction in Mice With Lupus Following Treatment With a Tolerogenic Peptide


Objective. Central nervous system (CNS) involvement in systemic lupus erythematosus (SLE) is manifested by neurologic deficits and psychiatric disorders. The aim of this study was to examine SLE-associated CNS pathology in lupus-prone (NZB × NZW)F₁ (NZB/NZW) mice, and to evaluate the ameliorating effects of treatment with a tolerogenic peptide, hCDR1 (human first complementarity-determining region), on these manifestations.

Methods. Histopathologic analyses of brains from lupus-prone NZB/NZW mice treated with vehicle, hCDR1, or a control scrambled peptide were performed. The messenger RNA expression of SLE-associated cytokines and apoptosis-related molecules from the hippocampi was determined. Anxiety-like behavior was assessed by open-field tests and dark/light transfer tests, and memory deficit was assessed using a novel object recognition test.

Results. Infiltration was evident in the hippocampi of the lupus-afflicted mice, and the presence of CD3+ T cells as well as IgG and complement C3 complex deposition was observed. Furthermore, elevated levels of gliosis and loss of neuronal nuclei immunoreactivity were also observed in the hippocampi of the mice with lupus. Treatment with hCDR1 ameliorated the histopathologic changes. Treatment with hCDR1 down-regulated the high expression of interleukin-1β (IL-1β), IL-6, IL-10, interferon-γ, transforming growth factor β, and the proapoptotic molecule caspase 8 in the hippocampi of the mice with lupus, and up-regulated expression of the antiapoptotic bcl-xL gene. Diseased mice exhibited increased anxiety-like behavior and memory deficit. Treatment with hCDR1 improved these parameters, as assessed by behavior tests.

Conclusion. Treatment with hCDR1 ameliorated CNS pathology and improved the tested cognitive and mood-related behavior of the mice with lupus. Thus, hCDR1 is a novel candidate for the treatment of CNS lupus.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by dysregulated T cell and B cell immune responses that are associated with clinical manifestations involving multiple organ systems (1). A synthetic peptide, hCDR1 (human first complementarity-determining region) (2), based on the CDR1 sequence of an autoantibody (3), ameliorated lupus manifestations in models of both spontaneous and induced SLE (4). Thus, hCDR1 down-regulated anti–double-stranded DNA autoantibody levels, proteinuria, and the formation of immune complex deposits in the kidneys, resulting in better survival rates for the treated mice (4). Treatment with hCDR1 reduced production of the “pathogenic” cytokines (i.e., interleukin-1β [IL-1β], tumor necrosis factor α [TNFα], interferon-γ [IFNγ], and IL-10), whereas expression of the immunosuppressive cytokine transforming growth factor β (TGFβ) was up-regulated (4). The mechanisms underlying the beneficial effects of hCDR1 involve inhibition of T cell receptor signaling following its binding to class II major histocompatibility complex (5), the induction of...
CD4+CD25+ Treg cells (6), and the reduction of apoptosis rates (7,8).

Neuropsychiatric (NP) syndrome is one of the manifestations reported in patients with SLE (9,10). Active kidney disease and central nervous system (CNS) involvement are the most frequently observed causes of death in patients with SLE (11). Elevated levels of brain-reactive antibodies were detected in the serum or cerebrospinal fluid (CSF) of patients with NPSLE (12,13). Double-stranded DNA–specific antibodies that cross-react with NR2, a subunit of the N-methyl-D-aspartate receptor on neuronal cells, were shown to cause neuronal death in vivo and in vitro (14). Elevated levels of NR2-specific autoantibodies were also observed in the sera of patients with SLE (15) and were reported to cause neuronal damage and memory deficit via access to the CNS (16). Hippocampal atrophy and biochemical signs of neuronal and astrocytic damage were reported in SLE patients with CNS involvement (17,18).

(NZB × NZW)F1 (NZB/NZW) mice in which lupus develops spontaneously (19) exhibit CNS involvement manifested by the presence of antineuronal antibodies in the brain (20) and changes in cognitive function (21). Neuropathology in mice with lupus involves disruption of the blood–brain barrier, consequently allowing infiltration of large molecules and immune cells, which is normally restricted (22). The latter is accompanied by deposition of immune complexes, complement activation (23), and induction of proinflammatory cytokines (24) that have been implicated in the pathogenesis of SLE. Behavioral changes were reported in lupus models and were found to correlate with hippocampus aberrations (25–28).

In the present study, we investigated CNS manifestations in NZB/NZW mice with lupus and their association with behavior deficits. Furthermore, we assessed the effects of treatment with the tolerogenic peptide, hCDR1, on these manifestations. We observed cell infiltration, immune complex deposits, gliosis, loss of neuronal nuclei immunoreactivity, and an altered cytokine profile in the hippocampi of mice with lupus. These manifestations were accompanied by behavioral abnormalities. Treatment with hCDR1 ameliorated the CNS manifestations and improved the behavioral performance of the mice with lupus.

MATERIALS AND METHODS

Mice. Female NZB/NZW mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were handled according to protocols approved by the Weizmann Institute Animal Care and Use Committee, using international guidelines.

Peptides and treatment. The synthetic peptide hCDR1 (2), which has a sequence (GYYWSWIROPPGKGEWIG) based on the CDR1 of the human monoclonal autoantibody (3), was synthesized at Polypeptide Laboratories (Torrance, CA). A peptide containing the same amino acids as hCDR1, with a scrambled order, designated scrambled peptide (SKGIPOYGWPWGFRYEI), was used as a control. Captisol (sulfobutylether β-cyclodextrin; CyDex, Lenexa, KS) was used as a vehicle. Eight-month-old NZB/NZW mice were injected subcutaneously with hCDR1, scrambled peptide (both at a dose of 50 μg per mouse), or vehicle alone, once weekly for 10 weeks.

Brain histology. The brain hemispheres of the mice were fixed with 4% paraformaldehyde. Serial sagittal sections from the lateral 1.08–1.68-mm area were prepared. Frozen cryostat (20 μm) or paraffin-embedded (4 μm) sections were used. All treatment experiments consisted of 6–8 mice/group; 2 sections from each mouse were analyzed. All regions within the sections were examined. All data presented are based on 3–5 independent experiments. Histopathology was evaluated by 2 examiners who were blinded to the treatment groups.

Hematoxylin and eosin (H&E) staining. Sections were stained with Mayer’s hematoxylin solution (Finkelman, Yehud, Israel). Slides were analyzed using a light microscope (Nikon Eclipse E800). The infiltration index, determined by H&E staining, was graded as follows: 0 = no infiltration, 1 = low level of infiltration, 2 = moderate level of infiltration, and 3 = high level of infiltration.

Staining for CD3+ T cells. Sections were incubated with a rat anti-mouse CD3+ antibody (Serotec, Raleigh, NC) and then incubated with biotin-conjugated goat anti-rat IgG (Chemicon, Temecula, CA) followed by incubation with Cy3-conjugated streptavidin (Jackson ImmunoResearch, West Grove, PA) and Hoechst stain (added in all immunofluorescence procedures) (Molecular Probes, Eugene, OR). Slides were mounted in Aqua-Poly/Mount (Polysciences, War- rington, PA) and analyzed with a fluorescence microscope (Nikon Eclipse E800), using Nikon ACT-1 software.

IgG and complement C3 immune complex deposits. Sections were incubated with fluorescein isothiocyanate (FITC)–conjugated goat anti-mouse IgG (Jackson ImmunoResearch) or with complement C3 FITC-conjugated goat anti-mouse IgG (ICN Pharmaceuticals, Costa Mesa, CA). The stained sections were graded as follows: 0 = no deposits, 1 = minimal level of deposits, 2 = moderate level of deposits, and 3 = high level of deposits.

Neuronal nuclei and glial fibrillary acidic protein (GFAP) staining. Sections were incubated with mouse antineuronal nuclei (Chemicon) or with rabbit anti-GFAP antibodies (Dako, Glostrup, Denmark). The secondary antibodies used were Cy3-conjugated goat anti-rabbit antibodies (Jackson ImmunoResearch) for GFAP or biotin-conjugated anti-mouse IgG (Vector, Burlingame, CA) for neuronal nuclei. FITC-conjugated streptavidin (Jackson ImmunoResearch) was added thereafter. A semiquantitative gliosis score based on GFAP staining was graded as follows: 0 = no gliosis, 1 = low level of gliosis (up to 10% of the area evaluated), 2 = moderate level of gliosis (up to 50% of the area evaluated), and 3 = high level of gliosis (>50% of the evaluated area). A
Semiquantitative score for neuronal nuclei loss was graded as follows: 0 = no loss, 1 = minimal loss (up to 30% of the brain sections), 2 = moderate loss (30–90% of the brain sections), and 3 = massive loss of neuronal nuclei staining (>90% of the brain sections). In addition, identical fields from the same area of the brain sections were photographed and computed using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). Ten different square areas (600 μm² each) were measured in the dentate gyrus region of each section. The intensity threshold was set to exclude nonspecific background fluorescence and was applied to all sections analyzed. The integrated optical density (IOD) was determined using the following equation: average intensity × area.

Electron microscopy. The mouse brain hemispheres were fixed with 3% paraformaldehyde and 2.5% glutaraldehyde. Ultrathin sections (70 nm) were prepared, analyzed under 120 kV using a Tecnai 12 transmission electron microscope (FEI, Hillsboro, OR), and digitized with a MegaView III CCD camera (Olympus, Lake Success, NY) using AnalySIS software (Nikon, Düsseldorf, Germany). Electron microscopy was performed at the Irving and Cherna Moskowitz Center for Nano and Bio-Nano Imaging at the Weizmann Institute of Science.

Flow cytometry. Splenocytes (1 × 10⁶) were incubated with anti-CD4–specific antibodies (Southern Biotechnology, Birmingham, AL), anti-TGFβ–specific antibodies (IQ Products, Groningen, The Netherlands), or anti-IFNγ–specific antibodies (eBioscience, San Diego, CA) and analyzed by flow cytometry. For intracellular staining, cells were fixed and permeabilized.

Real-time reverse transcription–polymerase chain reaction (RT-PCR). Brain hippocampi from mice were isolated from mice in the different groups. Total RNA was prepared using TriReagent (Molecular Research Center, Cincinnati, OH). Complementary DNA was prepared, and real-time RT-PCR was performed using the LightCycler system (Roche, Basel, Switzerland), according to the manufacturer’s instructions. The following primer sequences were used (forward and reverse, respectively): for IL-1β, 5′-GAGAACCAAGCAACGC-3′ and 5′-GTGGTGATGTACAGCTTGC-3′; for IL-6, 5′-CCACTGGTGTCCTCCTC-3′ and 5′-AAGTGCCATCGTTGTTG-3′; for IFNγ, 5′-GAAGCTGACACTCGC-3′ and 5′-CTGGAGCTGTGGTTG-3′; for IL-10, 5′-ACCTGGTTCGGATCTCC-3′ and 5′-GCATTGTTCCCGTACCC-3′; for TGFβ, 5′-AGCGGACTACTATGCTAAAG-3′ and 5′-GTAAAGCAGGGAATTGT-3′; for Bcl-xL, 5′-GGACCGCGATCGAGCC-3′ and 5′-GCATTGTTCCCGTACCC-3′; for caspase 8, 5′-ACATAACCCAAACTCCTGCCAA-3′ and 5′-GTGGGATAGGATACAGCA-3′; for GFAP, 5′-AGCTAAGCTATCGC-3′ and 5′-GGCCCTTCTGACGGGA-3′; for β-actin, 5′-GTGACGTGTTGACATCCG-3′ and 5′-CAGTAAACAGTCCCGCT-3′. Values less than 0.05 were considered significant.

Western blot analysis. Lysates extracted from the hippocampi were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, as described previously (8). The membranes were incubated with antineuronal nuclei (Chemicon), anti-caspase 8 (Alexis Biochemicals, San Diego, CA), anti–Bcl-xL (Santa Cruz Biotechnology, Santa Cruz, CA), and antitubulin antibodies (Sigma-Aldrich, Poole, UK). Membranes were incubated with the second antibody coupled to horseradish peroxidase. Detection was performed using the enhanced chemiluminescence method. Protein expression was determined by photodensitometry, using the Image program (National Institutes of Health, Bethesda, MD).

Behavioral analyses. Behavioral analyses were performed at the end of treatment, before the mice were killed. One week before the behavior analyses, mice were placed in a system space with 12 hours of dark during daytime and 12 hours of light during the night. During experiment days, the mice were brought to the testing room for 1 hour of habituation before being subjected to the tests. Testing was conducted during the dark phase of the light/dark cycle.

Open-field test. The open-field apparatus consisted of a white plexiglass box (50 × 50 × 22 cm) with 16 squares painted on the floor. A lamp provided 120-lux illumination of the floor. Mice were placed individually in one corner of the open field for a 10-minute test session. Inner squares were defined as the center; the time spent in the center, the number of entrances to the center, and the total distance traveled were quantified manually or using a video tracking system (VideoMot2; TSE Systems, Bad Hamburg, Germany). The results presented were obtained from the video tracking system.

Light/dark transfer test. The light/dark transfer test was conducted in a square white plexiglass arena (50 × 50 × 22 cm) separated into 2 compartments. The “light compartment” was constructed of white plexiglass illuminated with 120 lux, and the “dark compartment” was made of black plexiglass and covered with black-topped plexiglass. Individual mice were placed into the dark compartment and allowed to explore the apparatus for 5 minutes. The transition between the light compartment and the dark compartment and the time spent in the illuminated side were measured by a video tracking system.

Novel object recognition test. The novel object recognition test was performed in the open-field apparatus (50 × 50 × 22 cm). Two days before the test, each mouse was subjected to a habituation session in the open field and explored the area without the presence of objects for 10 minutes. One day before the test, each mouse explored the area in the presence of 2 identical objects. On the experiment day, a novel (N) object replaced 1 of the 2 identical familiar (F) objects presented the previous day. Mice were placed in the field for 5 minutes, and the total time spent in exploration of each object was determined. The recognition index (RI) was determined as follows: RI = (N – F)/(N + F) × 100. Two unbiased observers recorded the data.

Statistical analysis. Statistical analysis was performed using the Mann-Whitney U test, the Kruskal-Wallis nonparametric test, Student’s t-test, and Spearman’s rank correlation test. P values less than 0.05 were considered significant.

RESULTS

Down-regulation of cell infiltration and immune complex deposition in the brains of mice with lupus following treatment with hCDRI. To investigate cell infiltration in the brains of NZB/NZW mice with lupus, the brains were extracted from mice that had been...
treated for 10 weeks with vehicle, hCDR1, or the control scrambled peptide (see Materials and Methods), stained with H&E, and analyzed for cell infiltration. Figure 1A shows representative images of brain sections from mice in the different groups. A prominent reduction in brain infiltration can be seen in the section from an hCDR1-treated mouse as compared with brain sections from vehicle-treated and scrambled peptide–treated mice (Figure 1A, parts a–c). Electron microscopy analysis revealed immune-cell infiltration of mononuclear cells (Figure 1A, part a, lower left inset). Figure 1A part e shows the results of analyses of infiltration in all brains tested. A significant decrease ($P < 0.0001$) in brain infiltration in hCDR1-treated mice in comparison with...
the vehicle-treated and scrambled peptide–treated mice was observed. Young, disease-free mice were excluded from the analysis, because no infiltration was observed in their brains (Figure 1A, part d).

In order to determine whether T cells were present in the brain infiltrates, immunohistochemical analysis, using an anti-CD3 antibody, was performed. Positively stained cells were counted (Figure 1B, part e). Figure 1B, parts a and c, show positively stained cells that were identified in the dentate gyrus area of the experimental mice that were treated with either vehicle or the scrambled peptide. The brains of hCDR1-treated mice were almost free of CD3+ T cells (Figure 1B, part b), as were the brains of the young control mice (Figure 1B, part d). The numbers of CD3+ cells in the hippocampi of the NZB/NZW mice, as shown in Figure 1B, part e, confirm the significant reduction in the number of CD3+ cells following treatment with hCDR1.

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Reduced gliosis and protection against neuronal nuclei immunoreactivity loss in the brains of mice with lupus following treatment with hCDR1. The inflammatory events in the brains of NZB/NZW mice might affect the status of glial cells and neurons. We therefore investigated expression of the astrocytic marker GFAP in the brains of mice with lupus in comparison with that in the brains of mice treated with hCDR1. Figure 3A, parts a, c, e, and g, and parts b, d, f, and h, show GFAP staining and the matching nuclear images, respectively. Representative brain sections from young control mice served as a baseline for normal GFAP distribution. Increased expression of GFAP was observed in the brains of vehicle-treated and scrambled peptide–treated mice (Figure 3A, parts a and e, respectively). Treatment with hCDR1 resulted in reduced GFAP expression (Figure 3A, part c) to levels similar to those seen in the young mice (Figure 3A, part g). Reduced gliosis in the hippocampi following hCDR1 treatment is shown as a reduction in the mean IOD (Figure 3A, part i), the mean semiquantitative gliosis score (Figure 3A, part j), and the

Figure 2. Treatment with hCDR1 (human first complementarity-determining region) down-regulates IgG and complement C3 immune complex deposition in the brains of lupus-afflicted (NZB × NZW)F1 mice. a–h, Representative IgG (a–d) and complement C3 (e–h) immune complex deposits in brain sections from vehicle-treated mice (a and e), hCDR1-treated mice (b and f), scrambled peptide–treated mice (c and g), and young control mice (d and h). The corresponding insets show nuclear (Hoechst) staining. Bar = 200 μm. i and j, levels of IgG and complement C3 deposits, respectively, in the brains from 6–7 mice in each group. Bars show the mean and SEM. * = P < 0.004; ** = P < 0.03, versus scrambled peptide.
mean GFAP gene expression, as measured by real-time RT-PCR (Figure 3B).

To determine the effect of lupus on the status of neurons in the brains of NZB/NZW mice, we stained brain sections with an antibody to neuronal nuclei and analyzed them for neuronal nuclei immunoreactivity. Figure 3C shows representative brain sections stained with neuronal nuclei–specific antibodies (parts a, c, e, and g) and nuclear staining (parts b, d, f, and h). A loss or decrease in neuronal nuclei immunoreactivity was observed in the brains of vehicle-treated and scrambled peptide–treated mice (Figure 3C, parts a and e, respectively), as compared with the brain of a young mouse (Figure 3C, part g). The loss of neuronal nuclei immunoreactivity was observed mainly in the hippocampus. Treatment with hCDR1 restored the immunoreactivity to neuronal nuclei (Figure 3C, part c). Double staining for neuronal nuclei and Hoechst staining (Figure 3C, parts a and b and parts e and f, respectively) revealed that the nuclei of the neuronal cells in the pyramidal cell layer and in the dentate gyrus were intact, although their hippocampi were negative for neuronal nuclei staining. Figure 3C, parts i and j, which present the mean IOD of neuronal nuclei staining and mean semiquantitative score for neuronal nuclei loss, respectively, confirm the significant differences between mice treated with hCDR1 and the control group.

Immunomodulation of proinflammatory cytokines and apoptosis-related molecules in the brains of NZB/NZW mice treated with hCDR1. Because cytokines play an important role in SLE, and because hCDR1 was shown to immunomodulate the cytokine profile (4), it was of interest to determine the status of the relevant cytokines in the brains of mice with lupus following treatment with hCDR1. Because most of the morphologic alterations in the brains of NZB/NZW mice with lupus (Figures 1–3) were mapped to the hippocampus, we extracted RNA from the hippocampi of mice from the different groups. Gene expression was determined by real-time RT-PCR. As shown in Figure 4A, the mRNA levels of IL-1β, IL-6, IFNγ, IL-10, and TGFβ were elevated in the hippocampi of vehicle-treated and
scrambled peptide–treated mice compared with the young control mice. Treatment with hCDR1 reduced the levels of these cytokines to those determined for the young control mice.

We sought to determine whether the downregulation of all of the above-mentioned cytokines tested in the hippocampi of hCDR1-treated mice was attributable to a decrease in the number of immune cells or was a result of cytokine immunomodulation by hCDR1. No significant differences were observed between the total numbers of spleen-derived CD4+ cells of vehicle-treated mice (mean ± SEM 27 × 10^6 ± 0.2) and hCDR1-treated mice (28 × 10^6 ± 0.8). However, as shown in Figure 4B, analysis of intracellular cytokine expression in spleen-derived CD4+ cells indicated that treatment with hCDR1 resulted in a significant increase in the expression of TGFβ, whereas the levels of IFNγ were reduced as compared with those in vehicle-treated mice.

It has been established that apoptosis plays a role in the pathogenesis of SLE (29). We therefore measured the mRNA expression of the proapoptotic molecule caspase 8, and that of the antiapoptotic molecule Bcl-xL, in the hippocampi of the experimental mice (Figure 4C). Protein levels of the apoptotic-related molecules were also measured (Figure 4D). The mRNA (Figure 4C) and protein (Figure 4D) levels of caspase 8 were up-regulated in vehicle-treated and scrambled peptide–treated mice as compared with the healthy controls, whereas treatment with hCDR1 diminished the expression of caspase 8 to levels similar to those observed in the young mice. In contrast, the gene expression (Figure 4C) and protein (Figure 4D) levels of Bcl-xL were up-regulated in hCDR1-treated mice compared with vehicle-treated and scrambled peptide–treated mice.

**Improvement in the behavior parameters of NZB/NZW mice with lupus following hCDR1 treatment.** We were interested in investigating whether the histopathologic findings in the hippocampi of the diseased mice were associated with impaired behavior, and whether treatment with hCDR1 ameliorated such behavior. To this end, after 10 weeks of treatment, female NZB/NZW mice were subjected to behavioral studies. First, in the open-field test, we monitored NZB/NZW mice that were treated with vehicle, hCDR1, or the scrambled peptide. The mean ± SEM total distances traveled were determined to be 1,313 ± 199 cm, 1,681 ± 316 cm, and 1,521 ± 216 cm for vehicle-treated, hCDR1-treated, and scrambled peptide–treated mice, respectively, suggesting no significant differences in the locomotion of the different groups. Young, disease-free mice were excluded, because their traveled distances were extremely different (data not shown).

Figure 5A shows that the mean percentage of time spent in the center (part a) and the mean number of entrances to the center (part b) in the open-field test were significantly higher among the hCDR1-treated mice (mean ± SEM 4.78 ± 1.50% and 7.07 ± 1.46, respectively) compared with the vehicle-treated, hCDR1-treated, and scrambled peptide–treated mice, respectively, suggesting no significant differences in the locomotion of the different groups. Young, disease-free mice were excluded, because their traveled distances were extremely different (data not shown).

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from the different groups are shown in Figure 5A, parts c–e. Furthermore, as shown in Figure 5B, an inverse correlation was observed between the mean time spent in the center during the open-field test (Figure 5B, part a) and the severity of infiltration in the hippocampi, as shown by the infiltration index (Figure 5B, part b) ($r = -0.48, P < 0.006$). Representative images of the hippocampi of individual mice from the different groups and the performance of these mice in the open-field test are shown in Figure 5C.

An additional test to assess anxiety-like behavior, namely, the light/dark transfer test, was performed. As can be seen in Figure 5D, hCDR1-treated mice spent more time in the light compartment of the box (mean ± SEM 4.41 ± 1.46% of the time) in comparison with vehicle-treated mice (1.87 ± 0.58%) and scrambled peptide–treated mice (1.65 ± 0.57%). Learning and memory performances of the experimental mice were assessed by the novel object recognition test. Figure 5E shows a significant reduction in the recognition index of vehicle-treated mice (mean ± SEM −27.55 ± 8.55) and scrambled peptide–treated mice (−1.45 ± 11.43) in comparison with hCDR1-treated mice (30.07 ± 8.76).

**DISCUSSION**

The main findings of the present study are that treatment with hCDR1 significantly ameliorated the brain pathology and behavioral abnormalities that developed in NZB/NZW mice with lupus. To the best of our knowledge, this is the first study showing significant beneficial effects on CNS manifestations in mice with lupus following treatment with a tolerogenic peptide.

Cell infiltration, including that of CD3+ T cells, was observed in the brains of the NZB/NZW mice with lupus, mainly in the hippocampi (Figure 1). Indeed, histopathologic findings in the brain that involved perivascular lymphocytic infiltrates were reported in patients with SLE (30) as well as in lupus-prone mice (23,31). Cell infiltration into the CNS could be attributable to the observed increased expression of adhesion molecules such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 (32). In agreement with those observations, treatment with hCDR1 down-regulated elevated levels of vascular cell adhesion molecule 1 in the hippocampus (Lapter S, et al: unpublished observations).

IgG deposition was reported in the choroid plexus of patients with SLE (33) and in the brains of MRL/lpr mice (23). Furthermore, elevated levels of complement C3 and C4 were observed in the CSF of patients with CNS lupus (34). Similarly, we demonstrated increased IgG and complement C3 deposition in the brains of NZB/NZW mice with lupus (Figure 2). Treatment with hCDR1 significantly down-regulated the deposition of both IgG and complement C3 immune...
complex in the brains of the treated mice, as previously demonstrated in the kidneys of diseased mice (4).

Upon their activation, astrocytes produce cytokines such as IL-6 (37). In accordance, in the present study, increased expression of the pathogenic cytokines IL-1β, IL-6, IL-10, and IFNγ was observed in the hippocampi of the diseased mice. Treatment with hCDR1 reduced the gene expression of those cytokines to the levels observed in young healthy mice (Figure 4A). Similar to our findings in NZB/NZW mice, IL-1β and IL-6 were shown to be up-regulated in the hippocampi of lupus-prone MRL/lpr mice (24) and in the CSF of SLE patients with neuropsychiatric involvement (38).

We previously reported that, whereas treatment with hCDR1 resulted in diminished secretion and expression of the pathogenic cytokines INFγ, IL-1β, TNFα, and IL-10 in the spleens and lymph node cells of mice with lupus, expression of the immunosuppressive cytokine TGFβ was up-regulated by treatment (4). In the present study, elevated expression of TGFβ was observed in the hippocampi of the diseased mice (Figure 4A). Similarly, high levels of TGFβ were detected in the CSF of patients with neuropsychiatric SLE (39). Treatment with hCDR1 significantly down-regulated the expression of this cytokine in the brains of the treated mice. It is noteworthy that expression of TGFβ protein and mRNA was elevated in other target organs, such as the kidneys, of the mice with lupus (40). Enhanced expression of TGFβ in target organs may lead to dysregulated tissue repair, progressive fibrogenesis, and eventually end-organ damage (40).

A significant observation was the loss of immunoreactivity for neuronal nuclei in the hippocampi of the mice with lupus and its restoration following treatment with hCDR1 (Figure 3C). It is noteworthy that nuclear staining (Hoechst) in negative neuronal nuclei neurons indicated that their nuclei were intact. In addition, no significant differences in the neuronal nuclei protein content were detected in the hippocampi of the different treatment groups (Figure 3D). The neuronal nuclei antigen was used as a marker to identify neuronal cell loss under various pathologic conditions (41, 42), and a decrease in neuronal nuclei immunoreactivity was suggested to be predictive of delayed neuronal degeneration (42). To the best of our knowledge, this is the first study showing the loss of neuronal nuclei immunoreactivity in the brains of mice with lupus.

Apoptosis has been shown to play a role in various autoimmune diseases, including SLE (43). Increased expression of mRNA and protein of the pro-apoptotic molecule caspase 8 and diminished expression of the antiapoptotic molecule Bcl-xL were observed in the hippocampi of the diseased mice. Up-regulated expression of caspase 3 in the brains of MRL/lpr mice suggested a caspase-dependent mechanism for apoptosis in these brains (44). Treatment with hCDR1 down-regulated the levels of caspase 8 and up-regulated expression of Bcl-xL. In addition, amelioration of the lupus manifestations in the NZB/NZW mice following treatment with hCDR1 was associated with diminished apoptosis, which was manifested by a decrease in caspase 8 expression and an increase in Bcl-xL expression (7, 8, 45).

SLE-induced hippocampal damage was reported to lead subsequently to behavioral dysfunction (46). In addition, structural hippocampal atrophy, as demonstrated by magnetic resonance imaging, was associated with the presence of cognitive dysfunction, especially memory, in patients with lupus (18). As in the case of patients with SLE, a link between neurodegeneration, behavioral dysfunction, and memory impairment was observed in lupus-prone mice (21, 46). In the present study, we observed increased anxiety-like behavior and memory impairment in the mice with lupus. Treatment with hCDR1 significantly improved the performance of the mice in all behavior tests in association with amelioration of the histopathologic findings in their brains (Figure 5).

Thus, treatment with hCDR1 led to significant amelioration of brain pathology in lupus-prone NZB/NZW mice, including improvement in the cognitive behavior of the treated mice. The beneficial effects of hCDR1 were specific, because treatment with a control scrambled peptide did not affect the diseased mice. Therefore, hCDR1 is a potential candidate for the specific treatment of CNS lupus.

**AUTHOR CONTRIBUTIONS**

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved
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