The mechanism by which a peptide based on complementarity-determining region-1 of a pathogenic anti-DNA auto-Ab ameliorates experimental systemic lupus erythematosus

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A peptide based on complementarity-determining region (CDR)-1 of a monoclonal murine anti-DNA Ab that bears the common idiotype, 16/6Id, was synthesized and characterized. The peptide, designated pCDR1, was found to be an immunodominant T-cell epitope in BALB/c mice. The CDR1-based peptide was shown to be capable of inhibiting the *in vivo* priming of BALB/c mice immunized with the peptide or with the whole anti-DNA 16/6Id$^+$ mAbs of either mouse or human origin. We show here that administration of pCDR1 (weekly, i.v., 100 μg/mouse) in aqueous solution for 5 weeks starting at the time of disease induction with the human 16/6Id prevented the development of clinical manifestations of experimental systemic lupus erythematosus (SLE). Further, 10 weekly injections of pCDR1 to BALB/c mice with an established experimental SLE down-regulated clinical manifestations of SLE (e.g., anti-DNA auto-Abs, leukopenia, proteinuria, immune complex deposits in the kidneys) in the treated mice. Prevention of SLE induction was shown to be associated mainly with a decrease in the levels of IL-2, INFγ, and the proinflammatory cytokine TNFα. On the other hand, the secretion of the immunosuppressive cytokine TGFβ was elevated. Amelioration of the clinical manifestations of an already established experimental SLE correlated with a dramatic decrease in TNFα secretion, elevated levels of TGFβ, and immunomodulation of the Th1 and Th2 type cytokines to levels close to those observed in healthy mice.

The induction of experimental systemic lupus erythematosus (SLE) has been previously reported in our laboratory and was achieved by using the human monoclonal anti-DNA Ab that bears the common idiotype, designated 16/6Id (1). This Ab could induce SLE in naive mice of different susceptible strains (2). The 16/6Id-induced disease resembles SLE in human and is manifested by high levels of auto-Abs, which include anti-DNA and antinuclear protein Abs as well as 16/6Id and anti-16/6Id specific Abs (1). The 16/6Id-immunized mice also develop lupus-associated clinical symptoms (e.g., leukopenia, proteinuria, and kidney damage). Experimental SLE can also be induced in mice after their immunization with either a murine anti-16/6Id mAb (3) or a murine anti-DNA 16/6Id+ mAb, 5G12 (4), suggesting the importance of the 16/6Id network in the disease. Furthermore, T-cell lines specific to the human anti-DNA 16/6Id$^+$ mAb were shown to be capable of inducing experimental SLE in syngeneic recipient mice indicating the role of T cells in the disease (5). Experimental SLE, although induced in mice that normally develop no symptoms of SLE, was found to share features with the SLE model of (NZBxNZW)F1 mice, which develop the disease spontaneously. Thus, sequencing of the variable regions coding for the heavy and light chains of anti-DNA mAb isolated from mice afflicted with experimental SLE show high homology with the variable regions of anti-DNA mAb isolated from (NZBxNZW)F1 mice (6).

Two peptides based on the sequences of the complementarity-determining regions (CDR) of the pathogenic murine monoclonal anti-DNA Ab (5G12) that bears the 16/6 Id were synthesized. pCDR1 and pCDR3 were shown to be immunodominant T-cell epitopes in BALB/c and SJL mouse strains, respectively, and induced a mild SLE-like disease in responder mice (7). Further, the CDR-based peptides inhibited the priming of lymph-node cells (LNC) of mice immunized with the same peptides or with the monoclonal anti-DNA 16/6Id$^+$ Abs of either mouse or human origin. The CDR1-based peptide was also shown to prevent auto-Ab production in BALB/c neonatal mice that were immunized later with either pCDR1 or the pathogenic auto-Ab (7).

In the present report, the ability of the CDR1-based peptide to immunomodulate SLE induced in BALB/c mice was tested. We show here that pCDR1 is capable of either preventing or treating an already established SLE-like disease. A decrease in Th1-type (IL-2, INFγ) cytokines was observed when mice were treated for experimental SLE prevention, whereas the amelioration of disease manifestations in the treatment protocol was associated with a pattern of Th1 and Th2 cytokines similar to that observed in healthy mice. A significant down-regulation of the proinflammatory cytokine TNFα and an up-regulated secretion of the immunosuppressive cytokine TGFβ was demonstrated in mice treated for either the prevention or immunomodulation of experimental SLE.

Materials and Methods

Mice. Mice of the BALB/c inbred strain were obtained from Olac (Bichester, U.K.). Female mice were used at the age of 8–10 weeks, unless specified otherwise.

Synthetic Peptides. The peptide based on the CDR1 TGYYMQWVKQSPEKSLEWIG (pCDR1; the CDR is underlined) of the murine monoclonal anti-DNA 16/6Id$^+$ auto-Ab (mAb 5G12; ref. 4), was prepared with an automated synthesizer (Applied Biosystems model 430A) by using the company's protocol for *t*-butyloxycarbonyl strategy (8). A peptide synthesized in the reversed order of pCDR1 (rev pCDR1) was used for control.

mAbs. The human anti-DNA mAb that bears the 16/6Id (IgG1/κ) was previously described (9, 10). The Ab was secreted by hybridoma cells that were grown in culture and was purified by using a protein G-Sepharose column (Pharmacia).

Abbreviations: CDR, complementarity-determining region; Id, idiotype; LNC, lymph node cells; pCDR1, peptide based on the sequence of CDR1; SLE, systemic lupus erythematosus.

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Immunization and Induction of Experimental SLE. To induce experimental SLE, mice were immunized with 1–2 μg of the human mAb 16/6Id and boosted 3 weeks later (1).

Treatment with the CDR1-Based Peptide. For prevention of experimental SLE, mice were given pCDR1 or the reversed pCDR1 (control peptide) i.v. (100 μg/mouse) concomitant with immunization and were injected weekly thereafter for 5 weeks. Treatment of an established disease had started 3.5 months after disease induction with the 16/6Id, when clinical manifestations were already observed. Mice received 10 weekly injections of the CDR1-based peptide given i.v. or s.c. (100 μg/mouse). Reversed pCDR1 was administered as control. Both prevention and treatment experiments were performed three times each.

Detection of SLE-Associated Clinical and Pathological Manifestations. Proteinuria was measured semiquantitatively by using Combistix kit (Ames Division, Bayer Diagnostics, Newbury, U.K.). White blood cells were counted after a 10-fold dilution of heparinized blood in distilled water containing 1% acetic acid (vol/vol). For immunohistology analysis, frozen kidney sections (6 μm) were fixed and stained with FITC-conjugated goat Abs to mouse IgG (γ-chain specific; Sigma).

ELISA. For measuring anti-DNA Abs, 96-well Maxisorb microtiter plates (Nunc) were coated with either methylated BSA or polyL-lysine (Sigma). The plates were then washed and coated with either 10 μg/ml of denatured calf thymus DNA (Sigma) or λ-phage double-stranded DNA (Boehringer Mannheim, 5 μg/ml). After incubation with different dilutions of sera, goat anti-mouse IgG (γ-chain specific) conjugated to horseradish peroxidase (Jackson ImmunoResearch) was added to the plates, followed by the addition of the substrate, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (Sigma). Results were read by using an ELISA reader. Results of assays to determine single- and double-stranded DNA were found to be similar. For the determination of 16/6Id-specific Abs, plates were coated with 10 μg/ml of human 16/6Id, and Abs to nuclear proteins were detected by using precoated plates (Diamedix, Miami). The assays were carried out as above.

Induction of Cytokines Production. Mice that were immunized with the human 16/6Id and either treated or not with the CDR1-based peptide were killed at different periods during or after treatment with pCDR1. Splenocytes and LNC were harvested and incubated (5×10^6/ml) in the presence of the 16/6Id. Supernatants were collected after 48 and 72 h.

Detection of Cytokines in Supernatants. Measurements of IL-2, -4, -10, INFγ, and TNFα were performed by ELISA by using the relevant standards, capture and detecting Abs (PharMingen) according to the manufacturer's instructions. For detection of TGFβ, plates were coated with recombinant human TGFβ1 sRII/Fc chimera (R & D Systems), and the second Ab used was the biotinylated anti-human TGFβ1 Ab (R & D).The substrate solution used was TMB color Reagent (Helix Diagnostics, West Sacramento, CA), and enzyme activity was evaluated by using 570- and 630-nm filters.

Detection of Intracellular Cytokines. Single-cell suspensions of LNC were exposed to a Cytoperm kit (Serotec) according to the company's protocol. Thereafter, cells were incubated with the appropriate anticytokine–FITC-conjugated Ab. Cells were assessed by a FACScan cytometer, and the data were analyzed by using LYSIS software (Becton Dickinson).

Statistical Analysis. Mann–Whitney and *t* tests were used for statistical analyses of the data.

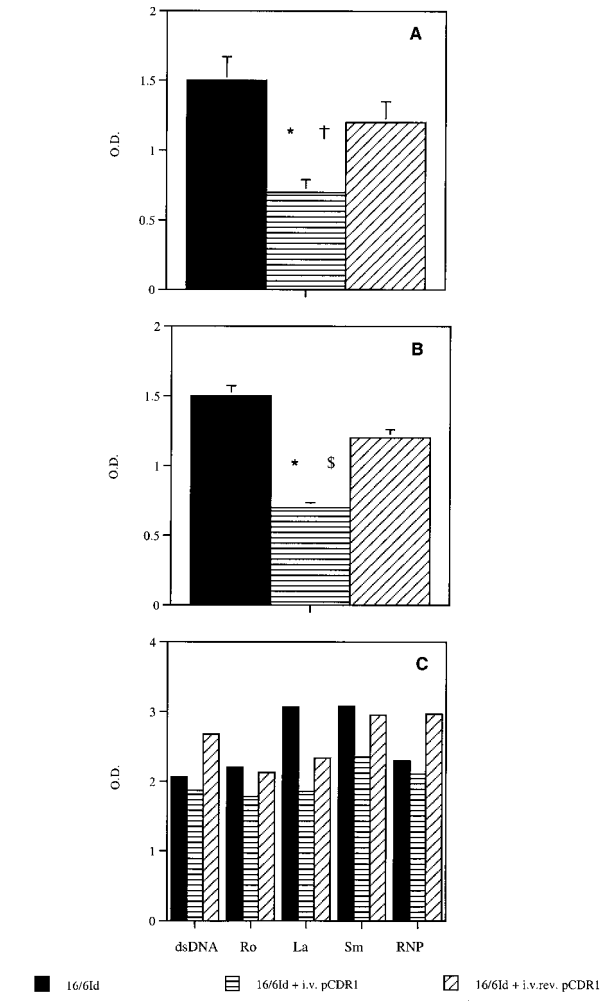

Fig. 1. Auto-Abs in BALB/c mice immunized with the 16/6Id. BALB/c mice (five mice per group) were immunized with 16/6 Id and concomitantly injected with pCDR1 or reversed pCDR1(100 μg/mouse i.v. once per week for 5 weeks) or were not treated. Results expressed as OD $\pm$ SD were obtained at the bleeding before sacrifice and represent all monthly bleedings. (*A*) Anti-DNA Ab titers measured on individual sera (dilution 1:1,000). (*B*) Anti-16/6Id Ab titers measured on individual sera (dilution 1:10,000). (*C*) Abs to nuclear antigens measured on pooled sera (dilution 1:100). OD levels of normal sera and of sera of mice that were injected only with pCDR1 were undetectable. The results are representatives of three experiments. (*A*) *, $P < 0.05$ as compared with reversed pCDR1-treated mice. †, $P < 0.01$ as compared with 16/6Id immunized untreated mice. (*B*) $, $P < 0.05$ as compared with 16/6Id immunized untreated or * 16/6Id immunized and reversed pCDR1-treated mice.

Results

Prevention of Experimental SLE. To find out whether the CDR1-based peptide is capable of preventing experimental SLE induced by the human anti-DNA 16/6Id, mice immunized with the latter were treated once per week for 5 weeks with pCDR1 (i.v. in PBS, 100 μg/mouse) or with a control peptide (reversed pCDR1), starting at the day of priming with 16/6Id. Fig. 1, which represents three similar experiments, demonstrates a decrease in the titer of anti-DNA (Fig. 1*A*), anti-16/6Id (Fig. 1*B*), and Abs to nuclear antigens (Fig. 1*C*) in the pCDR1-treated group compared with untreated or reversed pCDR1-treated groups. Table 1 shows that the clinical manifestations tested, namely leukopenia, proteinuria, and immune complex deposits in the kidneys, were also milder in the pCDR1-treated group of mice.

Table 1. The effect of treatment with pCDR1 on the clinical manifestations of experimental SLE

Immunization and treatment	WBC (mean ± SD)	Proteinuria (mean g/l ± SD)	Mean intensity of immune complex deposits ± SD
16/6 Id	2760 ± 391	1.4 ± 0.9	1.1 ± 0.2
16/6Id + reversed pCDR1	3220 ± 311	1.8 ± 1	0.88 ± 0.2
16/6Id + pCDR1	5950 ± 420*†	0.475 ± 0.35‡§	0.37 ± 0.1¶
pCDR1 only	5750 ± 208	0.225 ± 0.15	0.16 ± 0.1
Normal mice	5340 ± 313	0.18 ± 0.16	0.09 ± 0.09

BALB/c mice were immunized with 16/6Id and concomitantly injected with pCDR1 or reversed pCDR1 100 μg/mouse i.v. once per week for 5 weeks. Mice were followed for 8 months. Results of leukopenia and proteinuria were obtained 7 months after immunization and are representative of 3 experiments and of measurements performed at different time points. Results of immune complex deposits were evaluated as follows: 0 = no lesions or minimal lesions; 1 = moderate lesions; 2 = severe lesions. Kidney analyses were performed at death.
*, $P < 0.01$, ‡ and ¶, $P < 0.03$ compared to 16/6Id immunized mice that were not treated. †, $P < 0.01$ and §, $P < 0.03$ compared to 16/6Id immunized and reversed pCDR1 treated mice, respectively.

Fig. 2 demonstrates representative kidney sections of experimental mice. It can be seen in the figure that administration of pCDR1 prevented the formation of immune complex deposits in the kidneys. The effect of pCDR1 is specific because the control peptide, reversed pCDR1, did not affect specifically the auto-Ab titer (Fig. 1) and clinical manifestations, including kidney damage (Table 1, Fig. 2).

Treatment of Experimental SLE. It was of interest to find out whether the CDR1-based peptide is capable of down-regulating manifestations of experimental SLE when clinical symptoms are already observed. To this end, mice were immunized and boosted with the 16/6 Id and were followed for 3.5 months until clinical manifestations of the disease occurred. Groups of mice were then treated with 100 μg/mouse of pCDR1 administered either i.v. or s.c. once per week for 10 weeks. Fig. 3 demonstrates a significant decrease in the anti-DNA Ab titers in the pCDR1-treated groups (either i.v. or s.c., $P < 0.01$). Significantly reduced clinical manifestations (leukopenia, proteinuria, and immune complex deposits in the kidneys) were observed in the i.v.-treated group and to a lesser extent in the s.c.-treated group compared with the untreated group, as can be seen in Table 2. Fig. 4 represents immunohistology results of kidney sections of the different experimental groups. Both i.v. and s.c. administration protocols diminished the immune complex deposits in the pCDR1-treated groups. No such amelioration could be observed in the group of mice treated with the control peptide, the reversed pCDR1 (Table 2, Fig. 4). These results were reproducible in three independent experiments.

pCDR1 Down-Regulates Experimental SLE by Immunomodulating the Cytokine Profile. Because cytokines were shown to play a major role in the pathogenesis of experimental SLE (11), it was of interest to find out whether treatment with pCDR1 affects the cytokine profile of the treated mice. Hence, BALB/c mice that were injected with 16/6 Id and treated with pCDR1 were killed monthly, and their LNC were stained for intracellular cytokines. Table 3 represents results that were obtained 1 month after booster injection with the 16/6Id. A decrease in specific staining for IL-2 and INFγ could be observed in lymph node cells of mice of the pCDR1-treated group. A similar decrease could be observed 1 month later (data not shown). As can be seen in the table, no detectable changes could be observed in lymph node cells stained for IL-4 and -10. Matching results were obtained in a second independent experiment. We also examined secreted cytokines in supernatants of LNC and spleens of the experi-

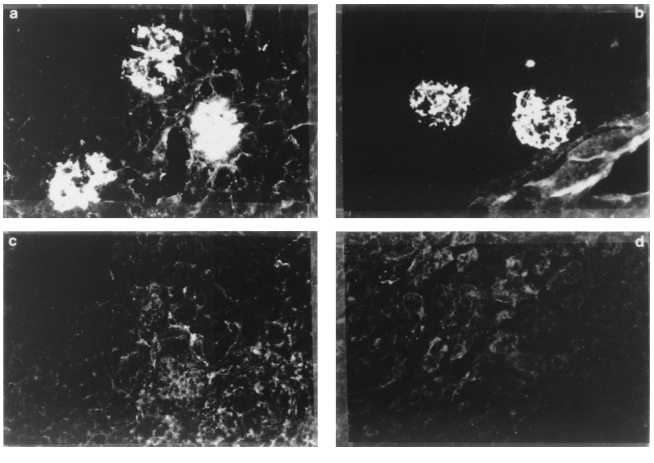

Fig. 2. Immunohistology of kidney sections of BALB/c mice that were treated with pCDR1 for prevention of experimental SLE. (*a*) 16/6Id-immunized mice; (*b*) mice immunized with 16/6 Id and concomitantly injected with reversed pCDR1; (*c*) 16/6Id-injected mice that were treated with pCDR1; (*d*) nonimmunized mice treated with pCDR1. Mice were killed 8 months after disease induction and their kidneys removed and analyzed for the presence of immune complex deposits as described in *Materials and Methods* (×400).

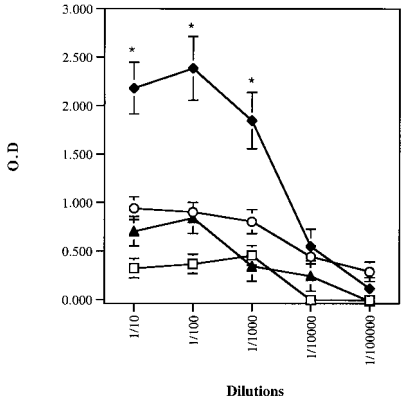

Fig. 3. Anti-DNA Abs in sera of SLE-afflicted BALB/c mice that were treated with pCDR1. BALB/c mice (20 mice/group) were immunized and boosted with 16/6Id. Later (3.5 months) mice were treated with pCDR1 i.v. or s.c., 100 μg/mouse once per week for 10 weeks. ◆, 16/6Id immunized; ○, 16/6Id immunized and treated with pCDR1 i.v.; ▲, 16/6Id immunized and treated with pCDR1 s.c.; □, sera of normal mice. Results expressed as OD ± SD were obtained at bleeding before death and represent all previous bleedings. Results were reproducible in three independent experiments. *, $P < 0.01$ as compared with both pCDR1-treated groups.

Table 2. Therapeutic effects of treatment with pCDR1 on the clinical manifestations of an established experimental SLE

Group	Immunization and treatment	WBC (mean ± SD)	Proteinuria (mean g/l ± SD)	Mean intensity of immune complex deposits ± SD
A	16/6Id	2870 ± 494	1.53 ± 1	1.5 ± 0.2
B	16/6Id + i.v. reversed pCDR1	3120 ± 701	1.67 ± 1.1	1.5 ± 0.5
C	16/6Id + i.v. pCDR1	6200 ± 490*	0.35 ± 0.37†	0.5 ± 0.3‡
D	16/6Id + s.c. pCDR1	5070 ± 625§	0.58 ± 0.36¶	0.66 ± 0.3**
E	Normal mice	7420 ± 511	0.06 ± 0.13	0

BALB/c mice were immunized with 16/6Id. Three and one half months after disease induction, they were treated with either pCDR1 (s.c. or i.v.) or reversed pCDR1 (i.v.) once per week for 10 weeks. Intensity of immune complex deposits were evaluated as described for Table 1. The above results were obtained at sacrifice (about 2 months after treatment had stopped).
*, $P < 0.01$ compared to group A and $P < 0.05$ compared to group B. ‡, $P < 0.01$ compared to group A and $P < 0.05$ compared to group B. †, $P < 0.02$ compared to groups A and B. ¶, $P < 0.02$ compared to groups A and B. ‡, $P < 0.03$ compared to group A. **, $P < 0.03$ compared to group A.

mental mice. Table 4 demonstrates the cytokine levels in the supernatants of 16/6 Id-stimulated LNC, about 2 weeks after the end of treatment (1 month after boosting with 16/6Id). As can be seen in the table, levels of proinflammatory cytokine TNFα as well as of IL-2 and INFγ were significantly lower in the group of pCDR1-treated mice, whereas secretion of the immunosuppressive cytokine TGFβ was elevated in these mice. The levels of IL-4 were below the detection sensitivity of the assay, whereas levels of IL-10 that were low in the 16/6Id-injected mice at the time of the assay were higher in the pCDR1-treated mice (260 pg/ml in supernatants of the pCDR1-treated mice compared with 120 and 140 pg/ml in 16/6Id-injected mice that were not treated or were treated with the control-reversed pCDR1, respectively). At a more progressed stage of the disease, when high levels of IL-10 were detected in supernatants of the SLE-afflicted mice (11), the concentration of the latter cytokine was lower in the pCDR1-treated mice and similar to that determined in normal mice (200 pg/ml in supernatants of pCDR1-treated mice as compared with 500 pg/ml and 460 pg/ml in supernatant of 16/6Id-injected and nontreated mice or reversed pCDR1-treated mice, respectively). Similar results were obtained when supernatants of splenocytes of the same mice were tested after their stimulation with the 16/6Id (data not shown).

We wanted to find out whether treatment with pCDR1 of mice with an already established experimental SLE affects the cytokine pattern as well. To this end, BALB/c mice with 16/6 Id-induced experimental SLE were treated after their clinical symptoms were observed. Two mice were killed monthly, and cytokines secreted by their LNC and spleen cells were assessed. Fig. 5 demonstrates the levels of cytokine secretion from spleens, at the end of treatment (about 6 months after disease induction), in comparison to cytokines in spleens of normal mice. A striking reduction in the levels of TNFα secreted by splenocytes of 16/6Id-immunized mice that either were not treated or were treated with the reversed CDR1-based peptide could be observed in supernatants of splenocytes of pCDR1-treated mice (either i.v. or s.c.). Both i.v. and s.c. treatment protocols increased significantly the levels of secreted TGFβ (Fig. 5). We have previously shown that at a progressed stage of the disease, the levels of secreted IL-2, INFγ, and IL-4 in the SLE-afflicted mice were lower than in healthy controls (11). Indeed, as can be seen in Fig. 5, treatment with pCDR1 (either i.v. or s.c.) resulted in the secretion of levels of the latter cytokines that are comparable to those determined in splenocytes of healthy mice. It is also shown in the figure that the secretion of IL-10 was immunomodulated by the treatment with pCDR1 to levels that are not substantially different from those of the normal mice. Similar results were obtained when cytokine secretion was measured in supernatants of LNC of mice of the different groups (data not shown). Thus, the beneficial effects of treatment with the CDR1-based peptide are associated mainly with the down-regulation of the proinflammatory cytokine TNFα that was shown to play a pathogenic role in SLE (11) and with an up-regulation in the secretion of the immunosuppressive cytokine TGFβ. The i.v. and s.c. treatment protocols had similar

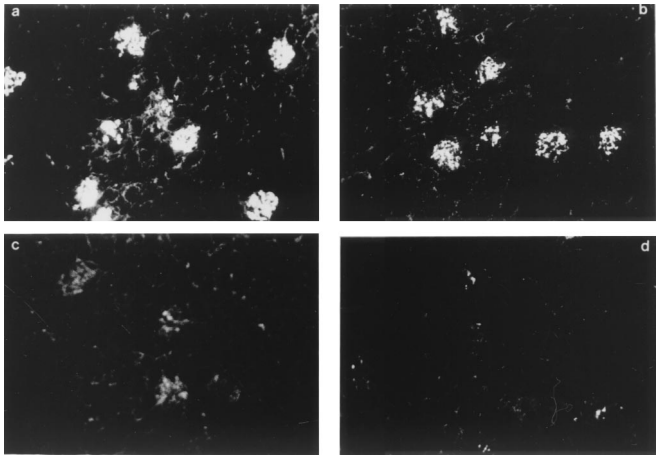

Fig. 4. Immunohistology of kidney sections of BALB/c mice treated with pCDR1 after clinical symptoms were observed. (*a*) 16/6Id immunized mice; (*b*) 16/6Id immunized mice treated with reversed pCDR1 i.v.; (*c*) 16/6Id immunized mice treated with pCDR1 s.c.; (*d*) 16/6Id immunized mice treated with pCDR1 i.v. Mice were killed 8 months after disease induction and their kidneys removed and analyzed for the presence of immune complex deposits (×20).

Table 3. Cytokine profile in BALB/c mice that were treated with pCDR1 for the prevention of experimental SLE

Cytokine	Intracellular staining: 16/6Id,* %	16/6Id + pCDR1, %	16/6Id + reversed pCDR1, %
IL-2	100	47	115
INFγ	100	56	81
IL-4	100	100	98
IL-10	100	99	98

Cytokine profile was determined by intracellular staining of lymph node cells (see *Materials and Methods*) of mice killed 1 month after booster injection with the 16/6Id (≈2 weeks after treatment). The results are representative of two experiments (5–12% variations were observed between experiments).
*Staining of lymph node cells of mice immunized with 16/6Id was considered as 100% (100% stained cells = 2,650, 2,950, 2,300, and 1,550 for IL-2, INFγ, IL-4, and IL-10, respectively).

Table 4. Cytokine profile in BALB/c mice that were treated with pCDR1 for the prevention of experimental SLE

Cytokine	Cytokine secretion		
	16/6Id, pg/ml	16/6Id + pCDR1, pg/ml	16/6Id + reversed pCDR1, pg/ml
IL-2	800 ± 141	<60	806 ± 137
INFγ	9,000 ± 816	267 ± 94	8,000 ± 2,160
TNFα	470 ± 30	<20	590 ± 10
TGFβ	2,350 ± 150	4,300 ± 300	2,900 ± 100

Secretion of cytokines was determined by ELISA (see *Materials and Methods*) of supernatants of LNC stimulated with the 16/6Id. Results are of mice killed 1 month after booster injection with the 16/6Id (≈2 weeks after treatment). The results are representative of two experiments.

effects on the clinical manifestations as well as on cytokine secretion.

Discussion

The main findings of the present report are that pCDR1 is capable either of preventing experimental SLE or of treating an already established SLE-like disease. The immunomodulation of disease manifestations was shown to be associated mainly with a significant down-regulation of the proinflammatory cytokine TNFα and with the up-regulated secretion of the immunosuppressive cytokine, TGFβ. Secretion of the Th1 type (IL-2 and INFγ) cytokines was diminished in mice treated with pCDR1 for the prevention of disease induction. In mice treated with pCDR1, when clinical symptoms were already established, Th1- as well as the Th2-type cytokines were immunomodulated to levels similar to those detected in healthy mice.

In the present study, the beneficial effects of pCDR1 were demonstrated in a model of experimental SLE. That pCDR1 injected only weekly five times in PBS during the immunization period with the 16/6Id for disease induction prevented disease development is of great significance, taking into consideration that the human anti-DNA 16/6Id used for disease induction is a multideterminant molecule. Indeed, the latter treatment led to beneficial effects on all measured clinical manifestations. It is noteworthy that the effect of pCDR1 was long-lasting because the mice were treated at the time of disease induction, and the beneficial effects were still observed at sacrifice (about 7–8 months after treatment had stopped).

Treating an already existing experimental SLE with pCDR1 is relevant for application to human disease, because in the latter case, treatment can start after patients are diagnosed as afflicted with SLE. Note that a relatively brief treatment regimen (10 weekly injections of 100 μg/mouse of the CDR1-based peptide) ameliorated all tested clinical manifestations of the complex systemic disease. The benefits of treatment with pCDR1 lasted for at least 2 months (mice were then killed) without further treatment. The CDR1-based peptide was also capable of preventing the lupus-like disease of (NZBxNZW)F1 mice (12) and, furthermore, it could down-regulate the clinical symptoms of an already developed disease in the latter SLE-prone mice and in MRL/lpr/lpr mice that also develop spontaneously SLE (H.Z., E.E., A. Meshorer, and E.M., unpublished work). The efficacy of the peptide based on the CDR1 of the murine 5G12 mAb in affecting the disease of (NZBxNZW)F1 mice is probably because of the high similarity between 5G12 mAB and anti-DNA Abs isolated from the SLE-prone mice (13, 14). Auto-Ab-derived peptides were recently shown to either delay disease onset, prolong survival, or ameliorate disease manifestations in (NZBxNZW)F1 mice (15–18).

It should be noted that both prevention and treatment with pCDR1 did not abolish completely the production of DNA-specific Abs. Nevertheless, a significant amelioration was determined in all of the clinical manifestations that were tested. These results are in agreement with our previous publications, in which experimental SLE was treated with methotrexate (19), tamoxifen (20), or methimazole (21). Beneficial effects of treatment of SLE-prone mice without complete depletion of measurable auto-Abs were reported by others as well (22–24).

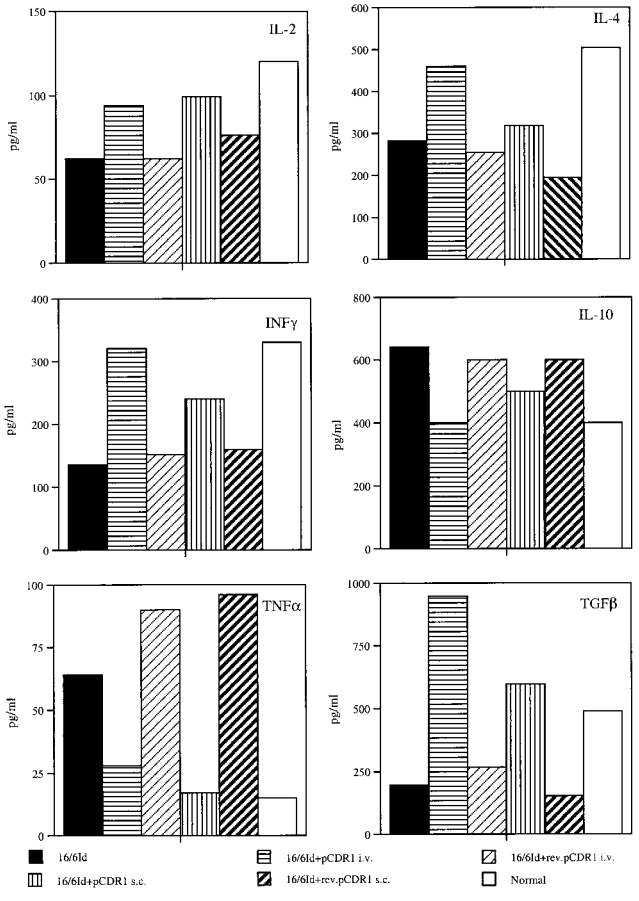

Fig. 5. The effect of treatment with the CDR1-based peptide on the cytokine pattern. BALB/c mice (20 mice/group) were immunized with 16/6 Id and 3.5 months later injected i.v. or s.c. with pCDR1. Two mice were killed monthly, and their spleen cells were stimulated with 16/6 Id. Supernatants were analyzed for cytokine secretion. Results are representatives of two experiments.

Cytokines have been suggested to play an important role in immune dysregulation observed in lupus-prone mice and in patients with SLE (25, 26). We have previously shown that the development of experimental SLE in mice involves two stages: first, increased production of Th1-type (IL-2, INFγ) followed by a significant increase in the secretion of Th2-type (IL-4, IL-10) cytokines (associated with decreased levels of both IL-2 and INFγ). Approximately 7 months after disease induction, when mice exhibit the full-blown disease, secretion of IL-2, INFγ, and IL-4 is diminished. High levels of the proinflammatory cytokines, TNFα and IL-1, are detected and maintained throughout disease course (11). A shift from Th1- to Th2-type cytokines has been reported in SLE patients (27), and it has been shown that both Th1- and Th2-type cells are down-regulated with disease progression in the patients (28).

A decrease in IL-2 and INFγ has been observed in mice treated with pCDR1 for prevention of SLE induction. The decline in the Th1-type cytokines was for a relative short period (about 2 months); nevertheless, it covered the period in which a

Th1 environment has been shown to be essential for induction of experimental SLE (11). INFγ plays a major role in the pathogenesis of SLE. Administration of INFγ along with disease induction aggravated disease manifestations (29). Further, MRL/lpr/lpr mice deficient of INFγ gene (30) or the INFγ receptor gene (31) were protected from disease development, as were (NZBxNZW)F1 mice treated with anti-INFγ Abs (32) or INFγ-soluble receptors (33). In addition to down-regulation of Th1-type cytokines, a reduction in TNFα and an increase in TGFβ were observed. Thus, a short course of pCDR1 administration resulted in a reduced production of the pathogenic cytokine TNFα, with a diminished production of IL-2 and INFγ and an increased secretion of TGFβ. The latter shifts in cytokine pattern resulted in the inhibition of disease development.

The beneficial effects of treating with pCDR1 mice with an established disease were associated with a significant decrease in the secretion of TNFα. This cytokine was reported to accelerate the kidney disease when injected to different experimental model animals (34); increased TNFα mRNA was observed in renal, splenic, and lung tissues of SLE-prone mice (25, 26), and high levels of soluble TNFα receptor were found in the sera of active SLE patients (35). Treatment of SLE-afflicted mice with either methotrexate or tamoxifen resulted in beneficial effects that were associated with a diminished secretion of TNFα (19, 20). Further, mice with SLE benefited significantly from treatment with either anti-TNFα or pentoxiphylline that was shown to reduce the levels of TNFα (36). It is very likely that pCDR1 modulates SLE manifestations by down-regulating TNFα production, which results also in restoration of the profile of Th1 and Th2 cytokines to levels similar to those observed in healthy mice (Fig. 5).

Treatment with the CDR1-based peptide resulted in a significant increase in the secretion of the immunosuppressive cytokine, TGFβ. Elevated levels of TGFβ were detected in mice that were treated with pCDR1 either for prevention or for curing an established disease. TGFβ-null mice were shown to develop autoimmune manifestations that resemble SLE (37), and the injection of a TGFβ cDNA expression vector into the skeletal muscle of the lupus-prone MRL/lpr mice decreased auto-Ab production (38). Both constitutive and stimulated levels of TGFβ are lower in patients with SLE, and the high IgG production seen in patients with SLE is attributed in part to low levels of TGFβ (39). It is not clear yet whether the elevated levels of TGFβ down-regulate the pathogenic cytokine TNFα or whether the administration of pCDR1 results in down-regulation of TNFα concomitant with an up-regulation of TGFβ. Nevertheless, the apparent effect of the immunomodulation of the above cytokines is a significant amelioration of the clinical manifestations of experimental SLE.

Treatment of SLE to date is not specific. The corticosteroids and immunosuppressive agents used to treat patients affect the function of the immune system and could be accompanied with severe adverse effects. The CDR1-based peptide, on the other hand, was shown to immunomodulate specifically experimental SLE that was induced by the pathogenic auto-Ab. It was also shown by us to affect beneficially the SLE-like disease that develops spontaneously in (NZBxNZW)F1 and in MRL/lpr/lpr mice. On the basis of its efficacy in the different models of SLE, pCDR1 might be considered a candidate for therapy of human SLE.

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