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Immunology

Modulation of murine systemic lupus erythematosus with peptides based on complementarity determining regions of a pathogenic anti-DNA monoclonal antibody

(autoantibodies/autoimmunity/immune tolerance/T-lymphocytes)

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ABSTRACT **Experimental systemic lupus erythematosus (SLE) can be induced in naive mice by immunization with a murine monoclonal anti-DNA antibody (mAb), 5G12, that bears a major idiotype designated 16/6 Id. Strain-dependent differences were observed in the proliferative responses of lymph node cells of mice immunized with two peptides based on the sequences of the complementarity determining region (CDR) 1 and 3 of mAb 5G12. The capacity of the peptides to bind to major histocompatibility complex class II molecules correlated with the proliferative responses. Immunization of high responder strains with the CDR-based peptides led to production of autoantibodies and clinical manifestations characteristic to experimental SLE. The CDR-based peptides could prevent autoantibody production in neonatal mice that were immunized later either with the peptide or with the pathogenic autoantibody. Furthermore, the peptides inhibited specific proliferation of lymph node cells of mice immunized with the same peptide, with mAb 5G12 or with the human mAb anti-DNA, 16/6 Id. Thus, the CDR-based peptides are potential candidates for therapy of SLE.**

Systemic lupus erythematosus (SLE) is a disease characterized by the production of autoantibodies to nuclear protein and nucleic acids, accompanied with clinical manifestations [e.g., leukopenia, thrombocytopenia, and kidney damage (1)]. We have previously demonstrated the induction of experimental SLE in naive mice of different strains following their inoculation with the human anti-DNA mAb carrying the 16/6 idiotype that was detected in sera of 54% SLE patients with active disease (2–4). The injected mice had high autoantibody levels that include anti-DNA and anti-nuclear protein antibodies, as well as 16/6 Id^+ and anti-16/6 Id antibodies, which indicate the activation of the 16/6 idiotypic network in those mice. We have further demonstrated that experimental SLE can be induced in mice of susceptible strains (BALB/c, SJL, and C3H.SW) following their immunization with either a murine anti-16/6 Id mAb (5) or a murine 16/6 Id^+ mAb, 5G12 (6). Experimental SLE, although induced in mice that normally do not develop any symptoms of SLE, was found to share features with (NZB × NZW)F_1 mice that develop the disease spontaneously. Thus, we have demonstrated high homology between the variable regions coding for the heavy and light chains of anti-DNA mAb isolated from mice afflicted with experimental SLE and the variable regions of anti-DNA mAb from (NZB × NZW)F_1 mice (7).

In the present article we report the synthesis and characterization of two peptides that were designed based on the sequences of the complementarity determining regions (CDRs) of a pathogenic murine monoclonal anti-DNA antibody (5G12) that bears the 16/6 Id. Those peptides were found to trigger T cell proliferation in various mouse strains, and induced, upon active immunization, the formation of autoantibodies and a mild experimental SLE in mice. Furthermore, when administrated in PBS into naive mice, the peptides were able to inhibit the production of autoantibodies and T cell activation in mice that were either immunized with these peptides or with the whole pathogenic antibody from a murine or human origin.

MATERIALS AND METHODS

Mice. The inbred mouse strains BALB/c and C57BL/6 were obtained from Olac (Oxon, U.K.). C3H.SW and SJL/J mice were obtained from The Jackson Laboratory. Female mice were used at the age of 8–10 weeks.

Synthetic Peptides. The CDR1-based peptide TGYYMQWVKQSPEKSLEWIG (pCDR1) and the CDR3-based peptide YYCARFLWEPYAMDYWGQGS (pCDR3) (the CDRs are underlined) were prepared with an automated synthesizer (Applied Biosystem model 430A) using the company's protocols for *t*-butyloxycarbonyl (BOC) strategy (8, 9). Peptide p307 of the human acetylcholine receptor α-subunit (10) and peptide p278 from the mouse heat shock protein 65 (11) were used a controls.

mAbs. The murine mAb 5G12 [IgG2a/κ; 16/6 Id^+ anti-DNA (6)] and 103 [IgG2a/κ; anti-(T,G)-A–L, (12)] were used. The human 16/6 anti-DNA mAb (IgG1/κ) was described (13, 14). Control human IgG were purchased from Sigma.

Immunization and Induction of Experimental SLE. Experimental SLE, was induced as described (3, 4). Briefly, mice were immunized with 1–2 μg of the human mAb 16/6, or with 10–20 μg of the murine mAb 5G12 or of the synthetic peptides emulsified in complete Freund's adjuvant (CFA). Three weeks later, the mice received a booster injection of the same dose of immunogen in aqueous solution.

Detection of SLE-Associated Clinical and Pathological Manifestations. Proteinuria was measured by a semiquantitative way, using Combistix kit (Bayer Diagnostics, Slough, U.K.). White blood cell were counted following a 10-fold dilution of the heparinized blood in distilled water containing 1% acetic acid (vol/vol). For immunohistology, frozen kidney sections (6 μm thick) were fixed and stained with fluorescein

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Abbreviations: APC, antigen-presenting cells; CDR, complementarity determining region; LNC, lymph node cells; MHC major histocompatibility complex; SLE, systemic lupus erythematosus; CFA, complete Freund's adjuvant.

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isothiocyanate-conjugated goat antibodies to mouse immunoglobulin G (γ-chain specific; Sigma).

ELISA. ELISA was done as described (6). For coating of plates, the following antigen concentrations were used: 5 μg/ml of rabbit-anti-16/6-Id Ig, 10 μg/ml denatured calf thymus DNA (Sigma), 10 μg/ml HeLa nuclear extract (15), and 10 μg/ml of the human 16/6 Id mAb.

Proliferation Responses of Lymph Node Cells (LNC). LNC of immunized mice (0.5×10^6/well) were cultured in the presence of different antigens as described (6). Following 4 days of incubation, [^{3}H]thymidine (0.5 μCi of 5 Ci/mmol; 1 Ci = 37 GBq; Nuclear Research Center, Negev, Israel) was added. Sixteen hours later, cells were harvested and radioactivity was counted.

Direct Binding of Biotinylated Peptides to Antigen-Presenting Cells (APC). N-terminal biotinylation of the peptides was performed in 0.1 M sodium bicarbonate solution at room temperature, with excess of biotinamidocaproate *N*-hydroxysuccinimide ester (Sigma) (16). Binding of biotinylated peptides to spleen cells was done as described (17). Three antibodies were used for inhibition of binding: 34–5-3 (anti-I-A^b; PharMingen), MKD6 (anti-I-A^d; Becton Dickinson), and 10.3.6.2 [anti-I-A^s (18)].

Neonatal Tolerance. BALB/c female mice, 24 hr old, were injected i.p. with 50 μl of 2 mg/ml peptide in PBS. Forty-eight hours later, the mice received an additional i.p. injection of peptide. The latter were immunized at the age of 6–8 weeks with the peptide or mAb 5G12 in CFA, as described above.

Inhibition of LNC Proliferation. For inhibition of LNC proliferation, mice were injected i.p. with 200 μg of peptide in PBS. In most experiments injection of peptides as inhibitors was performed concomitant with the immunization. Ten days after the priming, LNC were collected and proliferation carried out as described above.

RESULTS

LNC Responses to the CDR-Based Peptides. To test whether peptides based on the sequences of the CDR of mAb 5G12 can trigger T cell reactivity, mice were immunized with pCDR1 and pCDR3, as well as with mAb 5G12. Ten days after immunization lymph nodes were removed and tested for proliferation. Table 1 shows the proliferative responses of four mouse strains. As can be seen in Table 1, LNC from SJL, BALB/c, and C3H.SW mice responded to mAb 5G12 by proliferation. LNC of BALB/c mice immunized with pCDR1 proliferated very strongly in the presence of pCDR1 (Table 1) but not in response to pCDR3. On the other hand, pCDR3 could induce a significant proliferative response in SJL and C3H.SW mice. LNC of C57BL/6 mice, the only mouse strain found to be completely resistant to SLE induction by both 16/6 Id and anti-16/6 Id mAb (4, 5), did not proliferate significantly to either mAb 5G12 or to the two CDR-based peptides. No significant stimulation above background (SI = 1–1.4) was observed when the LNC were incubated in the presence of a control peptide, namely p278. Further, LNC of mice injected with p278 did not proliferate to either mAb 5G12 or to pCDR1 and pCDR3.

Table 1. Proliferative responses of LNC taken from mice immunized with mAb 5G12 or with the CDR-based peptides

Mice immunized with	Mouse strains			
	SJL	BALB/c	C3H.SW	C57BL/6
mAb 5G12	21	8.2	11	2.2
pCDR1	1	69	16.5	1.3
pCDR3	6	1.1	4.7	1.4

Results are expressed as mean stimulation index (cpm following stimulation/background cpm) of triplicates, and represent one experiment out of four experiments performed.

Binding of the CDR-Based Peptides to Live APC. To test the ability of the CDR-based peptides to bind to major histocompatibility complex (MHC) class II molecules, the biotinylated peptides were employed in binding assays using live APC. As seen in Table 2, both peptides, pCDR1 and pCDR3, were capable of binding to live APC of C3H.SW, SJL or BALB/c mice. Although both CDR-based peptides were capable of binding to APC of C3H.SW mice (H-2^b), no significant binding was detected to APC of the H-2 matched strain C57BL/6 (Table 2). It is also shown in Table 2 that most of the binding of peptides pCDR1 and pCDR3 to live APC of the various mouse strains was inhibited by the relevant anti-I-A antibodies, but not by nonrelevant antibodies.

Induction of Lupus-Associated Autoantibodies and Clinical Manifestations by Immunization of Mice with the CDR-Based Peptides. It was of interest to find out whether SLE could be induced using the above peptides. To this end, naive BALB/c and SJL mice were immunized with pCDR1 and pCDR3. The mice produced high anti-peptide antibodies, specific to the injected peptide (data not shown). In agreement with the LNC proliferative responses (Table 1), BALB/c and SJL mice were high responders to the peptides pCDR1 and pCDR3, respectively, producing high levels of anti-single-stranded DNA antibodies (Fig. 1). The relevance of these peptides to the 16/6 Id is supported by the demonstration (Fig. 1*b*, BALB/c; and *d*, SJL) that mice immunized with the CDR-based peptides produced anti-16/6 Id antibodies, as compared with mice injected with a control peptide ($P < 0.008$ for BALB/c, and $P < 0.01$ for SJL), although this reaction is lower than that observed following injection with mAb 5G12 (Fig. 1). As previously reported for 16/6 Id and mAb 5G12 immunized mice (3, 6), neither binding to nonrelevant antigens (e.g., BSA) nor increase in total Ig levels could be measured in sera of the peptide immunized mice.

Mice immunized with the peptides were also tested for clinical symptoms of SLE. As seen in Table 3, BALB/c and SJL mice immunized with peptides pCDR1 and pCDR3, respectively, developed proteinuria and leukopenia. In addition, as shown in Fig. 2, these mice developed moderate levels of immune complex deposits in their kidneys in contrast to mice immunized with the control peptide p278.

Neonatal Tolerance Induction Using the CDR-Based Peptides. In an attempt to induce tolerance to the pCDR1 peptide, neonatal BALB/c mice were injected i.p. with 100 μg of the peptide in PBS 24 hr and 72 hr after birth. In parallel, BALB/c mice were injected with a nonrelevant peptide, p307 (10). The tolerized mice were immunized at the age of 6 weeks with either peptide pCDR1 or mAb 5G12.

As can be seen in Fig. 3, mice tolerized with pCDR1 and immunized with either peptide pCDR1 or mAb 5G12 showed

Table 2. Binding of the CDR-based peptides to MHC class II on live splenic APC

Mouse strain	% binding		H-2	mAb used	% inhibition	
	pCDR1	pCDR3			pCDR1	pCDR3
BALB/c	45	41	d	anti I-A^d (MKD6)	76.7	100
BALB/c			d	anti I-A^b (34-5-3)	0	0
SJL	42	43	s	anti I-A^s (10.3.6.2)	100	92.8
SJL			s	anti I-A^d (MKD6)	0	0
C3H.SW	42	53	b	anti I-A^b (34-5-3)	60	84.4
C3H.SW			b	anti I-A^d (MKD6)	0	25
C57BL/6	9	8.5	b			
C57BL/6			b			

Splenic adherent cells were incubated for 16 hr at 37°C with the biotinylated peptides in the presence or absence of anti-Ia mAb, stained, and analyzed thereafter as described.

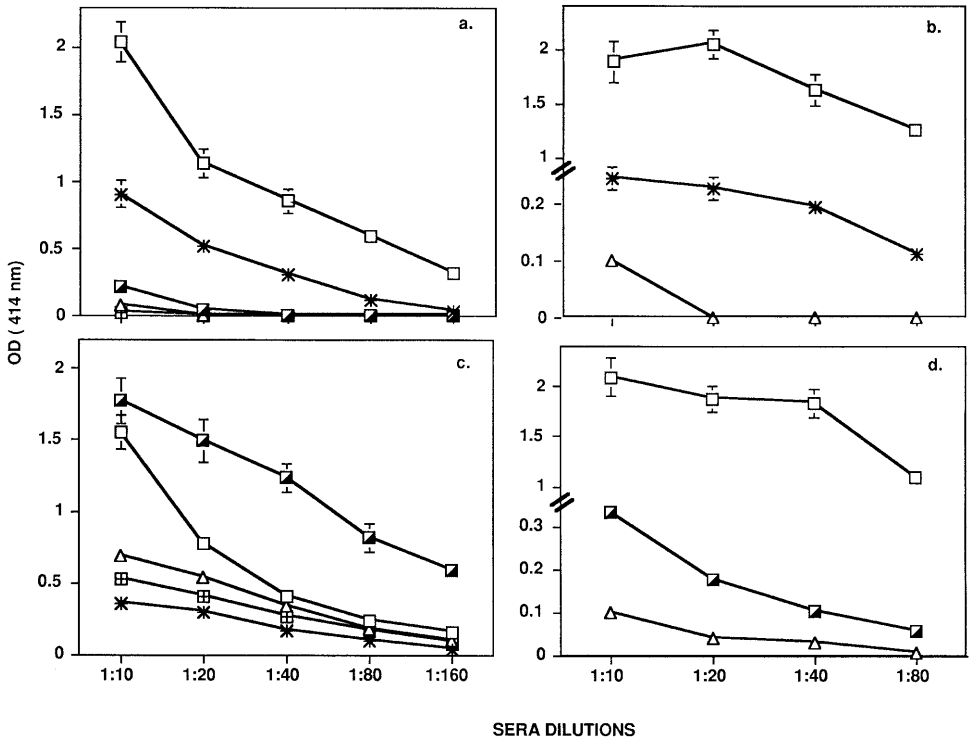

FIG. 1. Antibody levels in the sera of mice immunized with the CDR-based peptides. Sera of individual BALB/c (*a* and *b*) or SJL mice (*c* and *d*) immunized with either mAb 5G12 (□) or the peptides pCDR1 (∗), pCDR3 (◪), or p278 (△) taken 3 months after the booster injection, and sera of age matched naive mice (⊞), were tested for anti-single-stranded DNA (*a* and *c*) and anti-16/6 Id (*b* and *d*) antibody titers. Results are expressed as mean OD ± SD of each mouse group. No significant background binding to uncoated plates (OD ≤ 0.05) could be detected with all sera tested.

reduced titers of antibodies to either DNA (Fig. 3*a*) or nuclear proteins (Fig. 3*b*), in comparison to the mouse groups that were tolerized with the control peptide, p307. In addition, neonatal tolerance with peptide pCDR1 greatly reduced the levels of 16/6 Id^+ antibodies in the sera of mice immunized with mAb 5G12, as compared with mice tolerized with the control peptide (Fig. 3*c*).

Inhibition of LNC Proliferation with the CDR-Based Peptides. Next, we attempted to inhibit proliferation of LNC of mature mice using the CDR-based peptides as inhibitors. As demonstrated in Fig. 4*a*, administration of 200 μg of peptide pCDR1 i.p. into BALB/c mice blocked 80–90% of the proliferative response to that peptide in mice that were immunized with the peptide in CFA. A similar inhibition was observed when the peptide was injected 3 days prior to the day of challenge, at the day of challenge or when the CDR-based peptide was given twice. Likewise, as seen in Fig. 4*b*, injection of SJL mice with pCDR3, i.p., in PBS, inhibited by 80–90% the capacity of LNC to proliferate to that peptide. The control peptide p307 did not affect the proliferative responses of LNC of these mice (Fig. 4).

To test the effect of the CDR-based peptides on the immune response to the whole murine anti-DNA 16/6 Id^+ mAb (5G12), BALB/c mice were injected with peptide pCDR1, in PBS, while SJL mice were injected with peptide pCDR3, concomitant with their immunization with mAb 5G12 in CFA. Fig. 5 shows that proliferative responses of LNC to the immunizing mAb were significantly reduced (60% inhibition) when pCDR1 was injected i.p. to BALB/c mice, or if peptide pCDR3 was injected i.p. in PBS to SJL mice (85% inhibition). LNC of mice immunized with mAb 5G12 proliferated also in response to the appropriate immunodominant peptide (pCDR1 for BALB/c and pCDR3 for SJL mice), a response that was completely reduced when the relevant peptide was injected concomitant with the immunization with the antibody. In contrast, coinjection of a nonrelevant peptide, p307, did not affect the proliferative response to mAb 5G12 in either mouse strain (Fig. 5).

We have previously demonstrated cross-reactivity on the level of T cell responses between murine and human mAbs bearing the 16/6 Id (6). Therefore, we tested the ability of the CDR-based peptides of the murine mAb 5G12 to modulate the T cell reactivity to the human mAb 16/6 Id. Mice were immunized with the human mAb 16/6 Id in CFA concomitant with an i.p. injection of either pCDR1, pCDR3, or p307. As depicted in Fig. 6*a*, injection of peptide pCDR1 to BALB/c

Table 3. Clinical manifestations of mice immunized with the CDR-based peptides

	BALB/c		SJL	
Immunization	WBC	Proteinuria	WBC	Proteinuria
mAb 5G12	3,800 ± 400	0.975 ± 0.08	ND	0.8 ± 0.07
pCDR1	3,375 ± 350	0.88 ± 0.076	ND	0.375 ± 0.04
pCDR3	3,325 ± 400	0.30 ± 0.01	3,300 ± 1,343	0.9 ± 0.075
p278	6,470 ± 920	0.33 ± 0.02	7,150 ± 320	0.2 ± 0.025
Nonimmunized	6,800 ± 1,200	0.1 ± 0	8,100 ± 475	0.05 ± 0

WBC (white blood cell) measured as counts per mm^3. Proteinuria was measured as g/liter. ND, not done.

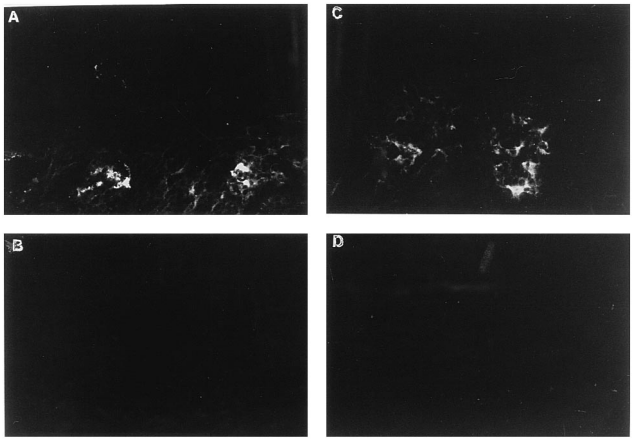

FIG. 2. Immunohistology of kidney sections from mice immunized with the CDR-based peptides. BALB/c (*A* and *B*) and SJL (*C* and *D*) mice were immunized with either pCDR1 (*A*), pCDR3 (*C*), or the control peptide p278 (*B* and *D*). Seven months later, mice were killed and their kidneys removed and analyzed for the presence of immune complex deposits as described. (×400.)

mice at the day of immunization with mAb 16/6, inhibited 90% of the proliferative response to the 16/6 Id. Similarly, pCDR3 inhibited the proliferative response of LNC of SJL origin to mAb 16/6 Id (Fig. 6*b*). Fig. 6 also demonstrates that LNC taken from BALB/c and SJL mice immunized with mAb 16/6 Id proliferated (SI of 2–4) in the presence of the immunodominant peptides of mAb 5G12, namely pCDR1 and pCDR3, respectively. The latter proliferative responses were also inhibited by the relevant CDR-based peptides.

DISCUSSION

In the present study, peptides based on the sequence of the CDRs of a pathogenic anti-DNA mAb (5G12) that bears the 16/6Id have been shown to be involved in both the induction of experimental SLE and the inhibition of the autoimmune responses.

Hahn and coworkers (19–21) found that a peptide of an anti-DNA mAb A6.1 isolated from NZB/W mice was involved in pathogenic and autoimmune processes in the mice. Thus, immunization of NZB/W mice with the latter CDR2 peptide, as well as with a cryptic peptide of the same antibody, increased plasma levels of anti-DNA IgG antibodies, accelerating immune complex deposition in the kidneys and nephritis (22, 23). The differences between the latter results and those of tolerance experiments reported here might be due to differences either in the peptides used or between the experimental model of induced SLE used by us and the spontaneous model of SLE studied by Hahn and coworkers (22, 23).

During the negative selection in the thymus, most of the T cells that recognize self-antigens are eliminated (24). Due to somatic mutations, peptides that represent the immunoglobulin CDR sequences are not always presented in the thymus, and as a consequence, T cells specific to those peptides may not be eliminated. A study utilizing a T cell line specific to a CDR2 peptide of a mAb showed that the line did not recognize the intact antibody (25). It was claimed that the cryptic CDR2 peptide is positively selected in the thymus (25). Indeed, the CDR2 in the latter antibody is encoded by a germ line gene sequence, and therefore exists in the thymus. In contrast, CDR1 and CDR3 of mAb 5G12 used in the present study are different from germ line gene sequences (7), and therefore it is highly probable that peptides pCDR1 and pCDR3 are not presented in the thymus.

Peptides pCDR1 and pCDR3 induced LNC proliferation in various mouse strains. Specifically, peptide pCDR1 was immunogenic in BALB/c mice, whereas peptide pCDR3 was immunogenic in SJL mice (Table 1). This pattern of proliferation may be explained by each of those peptides being the immunodominant peptides of mAb 5G12 in different mouse strains that are susceptible to the induction of experimental SLE, and may be the major contributors to the disease induction in that strain. The LNC taken from BALB/c mice that were immunized with mAb 5G12 were found also to proliferate significantly in the presence of peptides pCDR1 and pCDR3 (data not shown), suggesting that the mAb is processed to present at least those two peptides on the surface of APC. The latter is supported by our finding that MHC class II molecules on live APC isolated from C3H.SW, SJL, or BALB/c mouse strains were capable of binding both CDR-based peptides, pCDR1 and pCDR3 (Table 2).

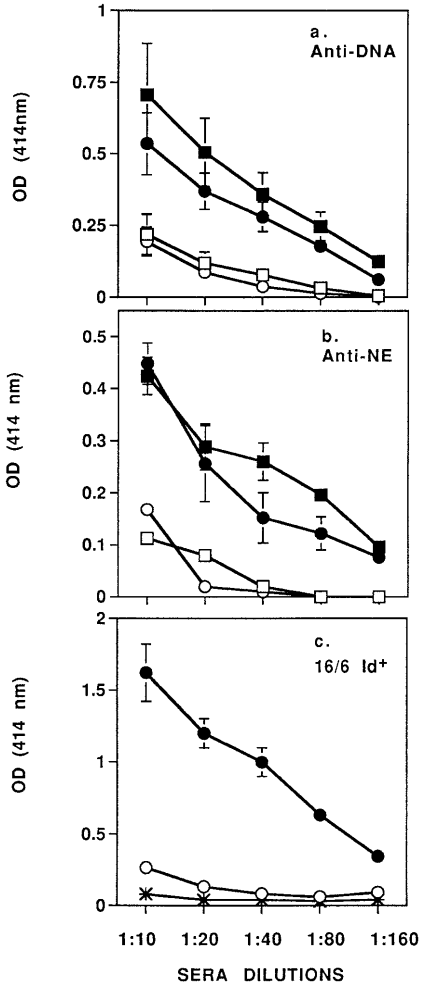

FIG. 3. Neonatal tolerization using the CDR-based peptides decrease autoantibody titers. Mice were tolerized and immunized as described. Two months following immunization, mice were bled and their sera subjected to ELISA experiments to test the levels of anti-single-stranded DNA (*a*), antinuclear antigens (*b*), and 16/6 Id^+ (*c*) specific antibodies. The groups include mice tolerized with peptide pCDR1 and immunized with either peptide pCDR1 (□) or with mAb 5G12 (○), mice tolerized with the nonrelevant peptide p307 and immunized with either peptide pCDR1 (■), or mAb 5G12 (●) and normal BALB/c mice (∗). Results are expressed as mean OD ± SD.

LNC of C57BL/6 immunized mice did not proliferate to the peptides, and proliferated only weakly to mAb 5G12 (Table 1), while the H-2 matched mouse strain, C3H.SW, responded by proliferation to both CDR peptides and to mAb 5G12. In addition, in contrast to C3H.SW mice, APC isolated from C57BL/6 mice did not bind biotinylated pCDR1 and pCDR3. The latter may be due to a processing defect in mice of the

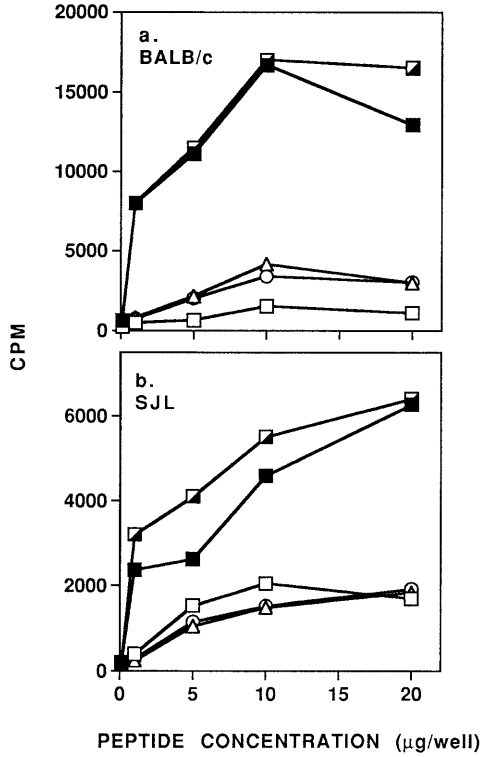

FIG. 4. *In vivo* inhibition of LNC proliferation. BALB/c (*a*) or SJL (*b*) mice were immunized i.d. in CFA with pCDR1 or pCDR3, respectively. The mice were also injected (i.p.) with 200 μg of the above immunizing peptides in PBS, either 3 days before immunization (□), at immunization day (○), or at both dates (△). Mice that were not treated (■), or treated with a control peptide, p307 (◪) were used as controls. LNC proliferation was then carried out as described. Results are expressed as mean cpm of triplicates. SD values did not exceed 10%.

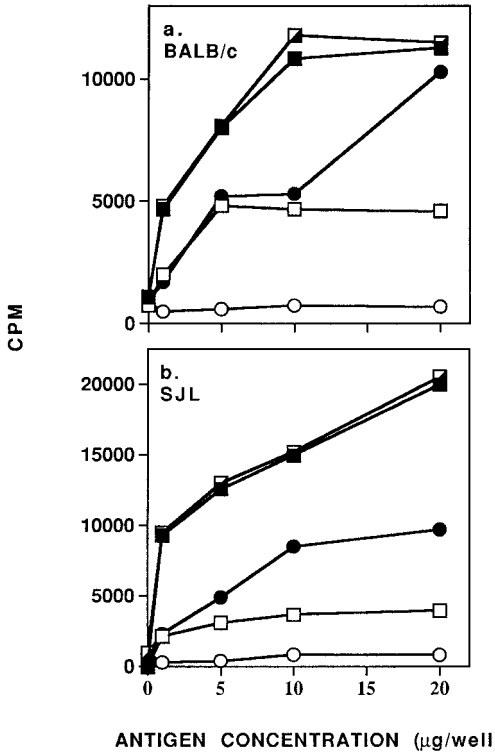

FIG. 5. LNC proliferative responses to mAb 5G12 in mice injected i.p. with the CDR-based peptides. LNC were taken from BALB/c (*a*) or SJL (*b*) mice treated with either pCDR1 (*a*) or pCDR3 (*b*). Proliferation is shown to mAb 5G12 of LNC taken from mice that were immunized and not treated (■), mice treated concomitant with immunization with peptide p307 (◪) or with the CDR-based peptides (□). Proliferation is also shown to the immunodominant CDR-based peptide of LNC taken from nontreated mice (●) or of mice treated with the peptide (○). LNC proliferation was then carried out as described. Results are expressed as mean cpm of triplicates. SD values did not exceed 10%.

C57BL/6 background, as was demonstrated recently for an hen egg lysozyme peptide (26). The inability of C57BL/6 to present the CDR-based peptides and to respond to the peptides by LNC proliferation may also explain the failure to induce experimental SLE in that mouse strain (4). It should be noted that as we have previously shown (17) APC of C57BL/6 mice are capable of binding efficiently a peptide that is a sequence of the human acetylcholine receptor α-subunit (p195–212). The resistance of the C57BL/6 mouse strain to the induction of experimental SLE was also observed following immunization with the human anti-Sm mAb 4B4, where anti-DNA antibodies were induced in BALB/c, but not in C57BL/6 mice (27). Thus, it appears that background genes of C57BL/6 can confer resistance to disease induction by autoantibodies.

It has been demonstrated that B cells are capable of presenting peptides from their own antibody to specific T cells on their MHC class II molecules (28, 29). LNC taken from SJL and C3H.SW mice immunized with pCDR1 were found to proliferate in the presence of peptide pCDR1 and also peptide pCDR3 (data not shown). We suggest, therefore, that following immunization with peptide pCDR1, B cells that produce an antibody that is similar or identical to the pathogenic mAb 5G12 may be activated. In turn, these B cells present a peptide similar or identical to pCDR3 and induce the formation of T cells specific to pCDR3. Therefore, in the induction of experimental SLE, the process of epitope spreading (30) may play an important role.

Peptides pCDR1 and pCDR3 induced autoantibody production as well as clinical manifestations including kidney damage. The latter corresponded to the ability of the peptides to trigger LNC to proliferate. These results may suggest that the initial trigger for induction of experimental SLE is a T cell response to the pathogenic moiety of the injected mAb.

In addition to the involvement of the CDR-based peptides in disease induction they were shown to be capable of inhibiting autoimmune responses. Thus, induction of tolerance to peptide pCDR1 in neonatal BALB/c mice inhibited autoantibody production following immunization with either peptide pCDR1 or mAb 5G12 (Fig. 3). Furthermore, administration of the CDR-based peptides in PBS could inhibit LNC proliferation to the peptides (Fig. 4), to the pathogenic parental murine mAb, 5G12 (Fig. 5), and to the original human anti-DNA 16/6 Id mAb (Fig. 6). The inhibition of the proliferation to the 16/6 Id may be due to the cross-reactivity on the T cell level between the murine 16/6 Id^+ mAb 5G12 and the human 16/6 mAb (6). The inhibition of serological manifestations as well as T cell proliferation by the CDR-based peptides suggest that they might be of potential for specific therapy of SLE.

Immunization by either pCDR1 or pCDR3 leads to the activation of peptide-specific T cells. Similarly, following injection of a pathogenic mAb, the latter is processed, and its CDR peptides are presented to specific T cells. This results in the formation of anti-peptide antibodies that in turn may cause the triggering of anti-self-antibodies and disease, or, the anti-peptide T cells induce directly the formation of anti-self-antibodies leading to disease.

Peptide pCDR1, when used to induce neonatal tolerance, could inhibit the formation of autoantibodies following immunization of BALB/c mice with either the peptide or mAb

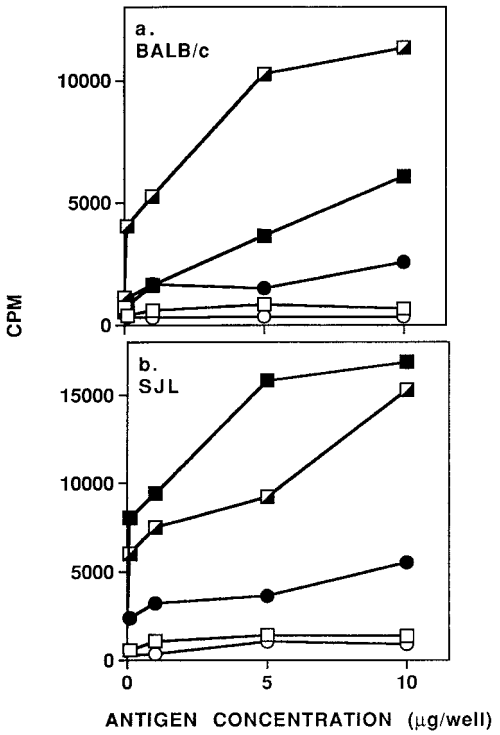

FIG. 6. LNC proliferative responses to the human mAb 16/6 Id in mice injected i.p. with the CDR-based peptides. Experiments were performed as described in the legend to Fig. 5.

5G12 (Fig. 3). The latter indicate that in BALB/c mice pCDR1 is a major T (Table 1) and possibly B cell epitope, and is a principal pathogenic moiety of the antibody. We also demonstrated that LNC proliferation to the dominant peptides or the whole antibody molecules could be abrogated by injection of the soluble peptides. The mechanism by which the soluble CDR-based peptides inhibited the LNC proliferation to the peptides or to the 16/6 Id^+ antibodies is not clear. We assume that the introduction of the immunodominant peptide in a soluble form inhibits the induction of newly activated T cells specific to it or to similar peptides, possibly by induction of anergy, blocking of epitope spreading, or shifting the cytokine profile of the activated T cells.

In conclusion, this study demonstrates that, without knowing the autoantigen for SLE, we have prepared peptides based on the CDR of a pathogenic autoantibody that can induce experimental SLE and also down regulate the autoimmune manifestations when used for tolerance in neonates or adult mice.

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