Antigen-Specific Therapy of Murine Lupus Nephritis Using Nucleosomal Peptides: Tolerance Spreading Impairs Pathogenic Function of Autoimmune T and B Cells[1]

Arunan Kaliyaperumal, Marissa A. Michaels, and Syamal K. Datta[2]

In the (SWR × NZB)F_1 mouse model of lupus, we previously localized the critical autoepitopes for nephritogenic autoantibody-inducing Th cells in the core histones of nucleosomes at aa positions 10–33 of H2B and 16–39 and 71–94 of H4. A brief therapy with the peptides administered i.v. to 3-mo-old prenephritic (SWR × NZB)F_1 mice that were already producing pathogenic autoantibodies markedly delayed the onset of severe lupus nephritis. Strikingly, chronic therapy with the peptides injected into 18-mo-old (SWR × NZB)F_1 mice with established glomerulonephritis prolonged survival and even halted the progression of renal disease. Remarkably, tolerization with any one of the nucleosomal peptides impaired autoimmune T cell help, inhibiting the production of multiple pathogenic autoantibodies. However, cytokine production or proliferative responses to the peptides were not grossly changed by the therapy. Moreover, suppressor T cells were not detected in the treated mice. Most interestingly, the best therapeutic effect was obtained with nucleosomal peptide $H4_{16–39}$, which had a tolerogenic effect not only on autoimmune Th cells, but autoimmune B cells as well, because this peptide contained both T and B cell autoepitopes. These studies show that the pathogenic T and B cells of lupus, despite intrinsic defects in activation thresholds, are still susceptible to autoantigen-specific tolerogens. ***The Journal of Immunology,*** **1999, 162: 5775–5783.**

Nucleosome-specific Th cells initiate and sustain the production of pathogenic anti-nuclear autoantibodies in systemic lupus erythematosus (SLE)[3] by a cognate interaction with autoimmune B cells (1–6). In lupus-prone (SWR × NZB)F_1 (SNF_1) mice, we have localized the critical autoepitopes for lupus nephritis-inducing Th cells in the core histones of nucleosomes at aa positions 10–33 of H2B and 16–39 and 71–94 of H4 (3). Autoimmune T cells of SNF_1 mice are spontaneously primed from early life to these disease-relevant epitopes (3). Moreover, immunization of preautoimmune SNF_1 mice with these nucleosomal peptide autoepitopes precipitates lupus nephritis by triggering Th1-type autoimmune T cells that drive anti-nuclear autoantibody production (3). Th2- and Th0-type cells are also involved in further maintenance of autoantibody production in lupus (1, 7). Herein, we investigated whether tolerization with the peptide autoepitopes we identified would affect the outcome of lupus, as has been the case in several organ-specific autoimmune diseases (reviewed in Ref. 8). However, unlike organ-specific autoimmune diseases where the autoimmune response is targeted against a restricted set of autoantigens and is mediated mainly by a select population of T cells, systemic autoimmunity in lupus involves a complex web of polyclonal T and B cell hyperactivity and multiple susceptibility genes (9–20). Therefore, it was unexpected that a brief tolerogenic regimen of the nucleosomal peptide epitopes administered into prenephritic, but autoimmune, SNF_1 mice could delay the development of lupus nephritis. Moreover, chronic tolerogenic therapy with the peptides administered into much older SNF_1 mice with established glomerulonephritis prolonged survival and even checked the progression of disease. Remarkably, the best therapeutic effect was obtained with the peptide $H4_{16–39}$, which had a tolerogenic effect on both autoimmune T and B cells.

Materials and Methods

Mice

NZB and SWR mice were purchased from Jackson Laboratory (Bar Harbor, ME). SNF_1 hybrids were bred at our animal facility. Female mice were used.

Abs

The following mAbs were used: anti-I-A^d (HB3), anti-I-$A^{b,d,q}$ (TIB120), anti-HSA (TIB183), anti-Thy1.2 (TIB99), anti-CD8 (TIB211), and anti-CD3 (145-2C11), all obtained from American Type Culture Collection (Manassas, VA).

Synthesis of peptides

All peptides were synthesized by F-moc chemistry (Chiron Mimotopes, San Diego, CA). The purity of the peptides was checked by amino acid analysis by the manufacturer. The nucleosomal histone peptides were $H4_{16–39}$, $H4_{71–94}$, and $H2B_{10–33}$ (3). We also used an I-A^d binding, 17-mer OVA ($OVA_{323–339}$) peptide that does not accelerate disease in SNF_1 mice upon immunization with CFA (3, 21). The peptides were purified by HPLC using a gradient of water and acetonitrile and were analyzed by mass spectrometry for purity.

Tolerance induction with histone-derived peptides in vivo

In long-term follow-up experiments, autoimmune but prenephritic SNF_1 females that were 12-wk-old (nine mice per group), were injected i.v. with either $H2B_{10–33}$, $H4_{16–39}$, $H4_{71–94}$, or $OVA_{323–339}$ peptide (300 μg/mouse) in saline. The control group received only saline. The animals received three more injections at 2-wk intervals (300 μg peptide/mouse each time).

Departments of Medicine and Microbiology-Immunology and Multipurpose Arthritis Center, Northwestern University Medical School, Chicago, IL 60611

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[2] Address correspondence and reprint requests to Dr. Syamal K. Datta, Arthritis Division, Ward 3-315, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611-3008. E-mail address: skd257@nwu.edu

[3] Abbreviations used in this paper: SLE, systemic lupus erythematosus; SNF_1, (SWR × NZB)F_1; CD40L, CD40 ligand.

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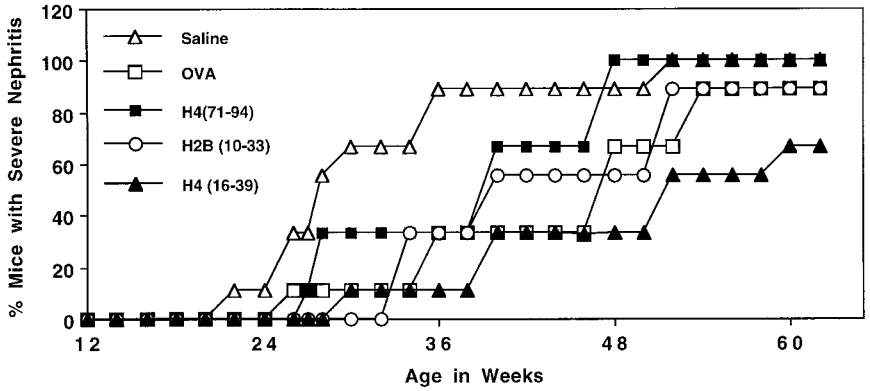

FIGURE 1. Effect of a brief therapy with nucleosomal histone peptides on spontaneous lupus nephritis. Incidence of severe (4+) lupus nephritis in SNF_1 mice (nine mice/group) that received four injections of the respective peptides or saline starting at 12 wk age. In this and subsequent figures OVA means the $OVA_{323–339}$ peptide.

The mice were monitored weekly for proteinuria using albustix (VWR Scientific, Chicago, IL) and killed when they developed persistent proteinuria (two consecutive weekly readings of 300 mg/dl or greater). Sera were collected for the determination of IgG anti-nuclear autoantibodies. Blood urea nitrogen was measured by azostix (Miles, Ekhart, IN). Kidney sections were stained with hematoxylin and eosin and anti-mouse Ig for the detection of immune complex deposition, and grading of glomerulonephritis by blinded observer was done as described (1, 14, 22–25). In short-term follow-up experiments to test the immunological consequences of the tolerance therapy early on, another batch of ~12-wk-old SNF_1 mice (nine per group) were treated as mentioned above, but they received the peptide injections every week for 4 wk. Two weeks after the last injection, these mice were killed for analysis of autoimmune T and B cells and grading of renal lesions. For chronic therapy of established glomerulonephritis, 18-mo-old SNF_1 mice with 300 mg/dl of persistent proteinuria were injected i.p. once a month with 300 μg peptide/mouse (six mice/group) until they were moribund and succumbed to renal disease.

Autoantibody quantitation

IgG-class autoantibodies to ssDNA, dsDNA, histones, and nucleosomes (histone/DNA complex), in culture supernatants or serum, were estimated by ELISA. Anti-DNA mAbs 564 and 205 were used to generate standard curves (1, 3, 14, 23). Sera were diluted 1:400 and heat-inactivated before use. Serum from normal SWR mice were used as negative control. Total polyclonal IgG levels were also measured by ELISA (1, 14, 23).

Isolation of $CD4^+$ T cells and B cells

Splenic $CD4^+$ T cells were isolated as reported earlier (1, 14). Briefly, splenic T cells were purified from 3- to 4-mo-old SNF_1 mice by nylon wool column followed by the lysis of $CD8^+$ T cells and contaminating B cells using anti-CD8 (TIB211), anti-Ia (TIB120), anti-HSA (TIB183), mAbs, and a mixture of rabbit and guinea pig complement (1:10) (Pel Freeze Biologicals, Rogers, AR). B cells were prepared from SNF_1 mice by treating splenocytes that had been passed through nylon wool with anti-Thy1.2 (TIB99) and complement twice.

Cytokine assays

Fresh splenic $CD4^+$ T cells (1×10^5/microwell) from tolerized or control mice were cocultured in triplicate with irradiated (3000 rad) anti-Thy-1.2 and complement-treated splenocytes (5×10^5/well) as APC (B cells plus macrophage preparation; Refs. 1 and 3) and different concentrations of "control" or "test" peptide in 200 μl final volume in HL-1 serum-free medium (BioWhittaker, Walkersville, MD) for 24–36 h in flat-bottom 96-well plates (Costar, Cambridge, MA). The culture supernatants were removed from wells after 24–36 h for cytokine assays (3). Anti-IL-2, anti-IFN-γ, and anti-IL-4 capture and biotinylated-revealing Ab pairs and the respective standards (rIL-2, rIL-4, rIFN-γ) were purchased from PharMingen (San Diego, CA). Streptavidin-HRP and the substrate tetramethylbenzidine were purchased from Sigma (St. Louis, MO). The cytokines were quantitated according to the manufacturer.

Helper assays for IgG autoantibody production

T cells (2.5×10^6/well) from the short-term follow-up batch of treated mice or T cells from unmanipulated SNF_1 mice were cocultured, respectively, with unmanipulated or tolerized SNF_1 splenic B cells (2.5×10^6/well) in 24-well plates for 7 days, as previously described (26, 27). Respective B cell preparations were cultured alone to measure baseline autoantibody production. Culture supernatants were collected, freeze-thawed, and assayed by ELISA for Abs against ssDNA, dsDNA, histones, and nucleosomes (histone/DNA complex). For studies involving stimulation of B cells by soluble CD40 ligand (CD40L), CD40L-CD8 fusion protein (28) was added at 1:4 dilution for the entire 7-day culture period. To further determine the fate of B cells in tolerized mice, B cells (2.5×10^6/well) were stimulated with 10 μg/ml of LPS or 10 μg/ml of anti-mouse Ig $F(ab')_2$ with and without rIL-4 (50 U/ml) in cultures.

Assay for regulatory T cells

To determine whether the tolerance therapy had induced any regulatory T cells, SNF_1 T cells (2.5×10^6/well) or purified $CD4^+$ or $CD8^+$ T cells (1×10^6/well) from the short-term follow-up batch of tolerized or saline-treated control mice were cocultured with a mixture of splenic B cells (2.5×10^6/well) and T cells (2.5×10^6/well) from unmanipulated SNF_1 mice in 24-well plates for 7 days. Culture supernatants were then collected, freeze-thawed, and assayed by ELISA for IgG Abs against ssDNA, dsDNA, histones, and nucleosomes.

Results

Brief therapy with nucleosomal histone peptides delays the development of spontaneous lupus nephritis

Twelve-week-old prenephritic SNF_1 females that did not have proteinuria (nine mice per group) were injected i.v. with either $H2B_{10–33}$, $H4_{16–39}$, $H4_{71–94}$, or $OVA_{323–339}$ peptide (300 μg/mouse) in saline, and the control group of mice received only saline. Each group of animals received three additional injections at 2-wk intervals. The mice were monitored weekly for proteinuria and sacrificed when they developed severe nephritis. By 22 wk, the control mice that received only saline started developing severe nephritis as documented by persistent proteinuria of 300 mg/dl or greater, and a 4+ grading of renal pathology (Fig. 1). At 28 wk of age, 55.5% of the saline control group, 33.3% ($p = 0.637$, Fisher's exact test) of the $H4_{71–94}$ peptide-injected group, and 11.1% ($p = 0.131$) of the $OVA_{323–339}$ peptide-injected group of mice developed severe nephritis, whereas the $H2B_{10–33}$ or $H4_{16–39}$ peptide-injected mice did not develop disease at this time ($p = 0.029$). The largest difference in incidence of severe nephritis between the peptide-injected groups and the saline control was from 36–38 wk of age. In the control group, 88.8% of the mice had developed severe disease, whereas the $OVA_{323–339}$, $H2B_{10–33}$, or $H4_{71–94}$ group had an incidence of only 33.3% ($p = 0.05$) and in the $H4_{16–39}$ group only 11.1% ($p = 0.003$) of the animals had developed severe nephritis (Fig. 1). Mice in all groups, except the $H4_{16–39}$ peptide-injected mice, developed severe nephritis by 54 wk of age. The $H4_{16–39}$ peptide-injected group had a 55.5% incidence of severe nephritis even at this advanced age, but the differences were not that significant ($p = 0.08$ compared with saline group; $p = 0.294$ compared with $OVA_{323–339}$ group).

Table I. *Incidence of lupus nephritis at sacrifice in the short-term batch of mice*

Group	Grading of Lupus Nephritis (percent incidence)[a]				
	0	1+	2+	3+	4+
Saline		33.3		33.3	33.3
$OVA_{323-339}$		66.6		33.3	
$H2B_{10-33}$		66.6	33.3		
$H4_{16-39}$	33.3	66.6			
$H4_{71-94}$			66.6	33.3	

[a] A separate batch of ~12-wk-old mice (nine mice/group) were treated with injections once a week for 4 wk. The mice were sacrificed when they were 22–23 wk old to evaluate their renal pathology.

T cell response to peptides in treated animals

In unmanipulated SNF_1 mice, T cells are spontaneously primed to the nucleosomal peptides early in life and respond to them in vitro (3). Therefore, T cells isolated at the time of sacrifice from peptide-treated or control mice in the long-term follow-up experiments (Fig. 1) were cocultured with APC in the presence of the peptides or nucleosomes, and their responses were measured by incorporation of [^{3}H]thymidine for proliferation and cytokine (IL-2, IFN-γ, and IL-4) production by ELISA. As these mice had already developed a 4+ grade of severe nephritis at the time of testing, the background levels of proliferation were high (data not shown). There was no deviation in cytokine production when the saline-treated group was compared with the peptide-treated groups (data not shown).

To test the immunologic consequences of the peptide therapy early on, before it is obscured by full-blown disease, another set of ~12-wk-old SNF_1 mice was injected with the various peptides or saline once a week for 4 wk and were killed 2 wk after the last injection. The animals were 22- to 23-wk-old at this time. We refer to this batch of mice as the "short-term" follow-up batch. At this earlier time point of sacrifice, the incidence and grading of renal pathology in this batch of mice are shown in Table I. Also in this batch of mice, we did not detect any consistent differences in cytokine production levels or cytokine profiles in T cells from the saline control vs the peptide-treated groups of mice in response to any of the peptides or to nucleosomes (data no shown).

Peptide therapy impairs CD4$^+$ T cell help for autoantibody production

The helper assay for autoantibody-inducing ability is a much more rigorous test for autoimmune Th function. Therefore, in additional experiments we used the helper assay to determine the function of T and B cells in tolerized animals from the short-term follow-up batch. To test the ability of T cells to functionally help B cells, CD4$^+$ T cells were isolated from the peptide-treated mice and cocultured with B cells isolated from unmanipulated 16- to 20-wk-old SNF_1 mice in a helper assay. The coculture supernatants were assayed for the presence of IgG Abs against ss-DNA, ds-DNA, nucleosomes, and histones. The results shown are the mean values ± SEM of five experiments (Fig. 2). Anti-dsDNA Ab production was reduced by ~50% in the cultures containing T cells from $OVA_{323-339}$, $H4_{71-94}$, and $H4_{16-39}$ peptide-injected groups in comparison to the saline-injected group ($p = 0.03$). The $H2B_{10-33}$ peptide-treated group showed a 5-fold decrease. Induction of anti-ssDNA Ab was also reduced by ~55% in the $H2B_{10-33}$ and $H4_{16-39}$ groups in comparison to the saline-injected group ($p = 0.005$–0.001), but reductions in the $OVA_{323-339}$ and $H4_{71-94}$ peptide-treated groups were not significant. Anti-nucleosomal Ab production showed a similar pattern. The cocultures with T cells from $H2B_{10-33}$, $H4_{16-39}$, and $H4_{71-94}$ peptide-injected groups produced 2.5- to 4-fold less anti-nucleosome autoantibody compared with that of the saline-treated control group ($p = 0.03$–0.05), but in the case of $OVA_{323-339}$ group the reduction was not significant ($p = 0.061$). The effect on anti-histone Ab induction, overall, was not as dramatic ($p = 0.1$).

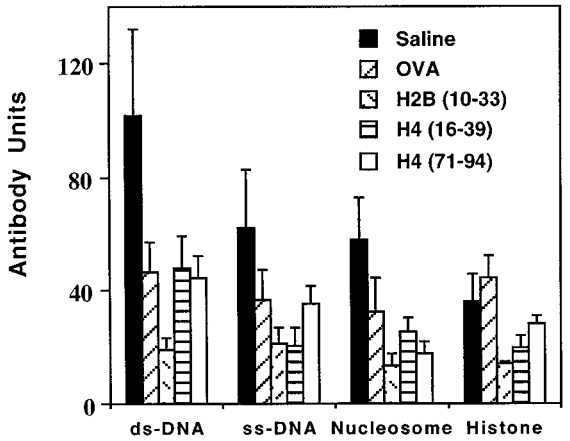

FIGURE 2. Ability of CD4$^+$ T cells from treated mice to help autoantibody production. Twelve-week-old SNF_1 mice were injected with respective peptides (300 μg/mouse) or saline every week for 4 wk. Two weeks after the last injection, CD4$^+$ T cells from the treated groups were cocultured with B cells from unmanipulated SNF_1 mice for 7 days. Units of IgG autoantibodies produced in the culture supernatants are expressed as mean ± SEM/dl from five experiments. Baseline levels of IgG autoantibodies produced by B cells cultured by themselves were: anti-dsDNA, 4.1 ± 0.8; anti-ssDNA, 2.2 ± 0.1; anti-nucleosome, 4.4 ± 1.1; and anti-histone, 3.7 ± 0.6.

The addition of rIL-2 to CD4$^+$ T cells does not lead to recovery of T cell help

To determine whether the diminished help by the CD4$^+$ T cells from peptide-treated mice were due to anergy or deletion, rIL-2 ranging from 12.5 to 100 U/ml was added to the helper assay cocultures (T cells from treated mice plus B cells from unmanipulated SNF_1 mice) at the beginning of the 7-day period. The saline-treated control mice produced 102 ± 15.5 U of anti-dsDNA Ab, and the addition of rIL-2 increased this concentration very little (Fig. 3). CD4$^+$ T cells from the $OVA_{323-339}$, $H4_{71-94}$, and $H2B_{10-33}$ peptide-treated mice did not show any improvement in their helping ability after the addition of rIL-2 (Fig. 3). The exception was the $H4_{16-39}$ peptide-treated group, where there was a modest recovery of help with an increase by 35% in anti-dsDNA autoantibody production after the addition of rIL-2 ($p = 0.05$), but the 30% increase in anti-ssDNA and 20% increase in anti-nucleosome Abs were not significant (Fig. 3).

The effect of peptide therapy on the ability of autoimmune B cells to receive T cell help

The effect of peptide therapy on immune function of the B cells was determined by coculturing the B cells from the peptide-treated mice with CD4$^+$ T cells from unmanipulated SNF_1 mice (3- to 4-mo-old) in the helper assay. When such T cell help was provided, the B cells from the peptide- and saline-treated groups did not show any difference in augmenting their ability to produce IgG autoantibodies, with the exception of the $H4_{16-39}$ peptide-injected animals, whose B cells still produced diminished levels of all autoantibodies in these cocultures. The level of anti-dsDNA Abs produced by B cells from $H4_{16-39}$ peptide-treated mice (45 ± 10.1

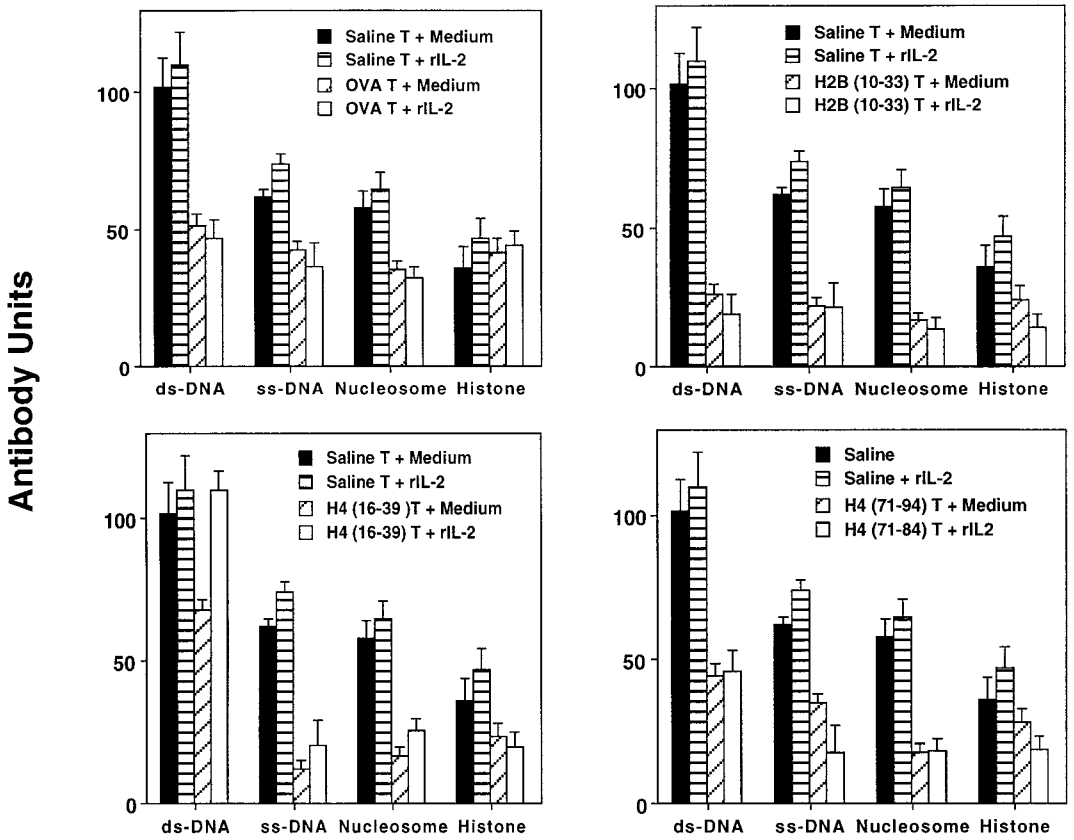

FIGURE 3. Effect of IL-2 on the ability of T cells to help autoimmune B cells. Experimental conditions were similar to Fig. 2. In the results shown here, 100 U/ml rIL-2 was added to the specified culture at the beginning of the 7-day period.

U/dl) was significantly reduced in comparison to saline-treated mice (160 ± 18.5 U/dl) ($p = 0.03$; Fig. 4). The production of anti-ssDNA Abs also showed a similar pattern: B cells from the saline-treated group produced 170 ± 10.9 U vs B cells from the $H4_{16-39}$ peptide-treated group, which produced 60 ± 7.9 U ($p = 0.005$). Production of anti-nucleosome Abs was reduced by 3-fold compared with controls (145 ± 20.8 vs 45 ± 5.9 U) in the $H4_{16-39}$ peptide-treated group, and anti-histone Ab production was reduced by ~4-fold ($p = 0.003$) in $H4_{16-39}$ peptide-injected animals as compared with the saline group. In the case of $H4_{71-94}$ peptide-injected animals, the production of anti-nucleosome ($p = 0.01$) and anti-histone ($p = 0.003$) autoantibodies, but not anti-dsDNA or anti-ssDNA autoantibodies, remained significantly low as compared with the saline group, even with the T cell help. The baseline autoantibody production by B cells cultured by themselves ranged from 1.3 to 2.5 U/ml in these experiments (Fig. 4).

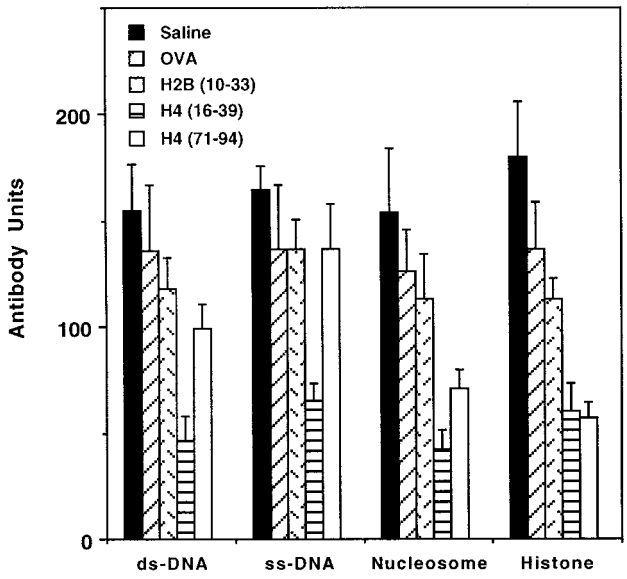

FIGURE 4. Effect of peptide therapy on ability of B cells to receive help. Mice were treated as mentioned in Fig. 2. B cells were purified from these mice and cocultured for 7 days with T cells isolated from unmanipulated SNF_1 mice. The units of IgG autoantibodies produced are expressed in mean ± SEM from five experiments. The baseline autoantibody levels produced by B cells cultured alone were: anti-dsDNA, 2.4 ± 0.96; anti-ssDNA, 2.1 ± 0.34; anti-nucleosome, 2.5 ± 1.9; and anti-histone, 1.3 ± 1.0.

The effect of anti-Ig F(ab′)$_2$ and rIL-4 on B cells of $H4_{16-39}$ peptide-treated mice

B cells from $H4_{16-39}$ peptide-treated animals were less responsive to help from T cells of unmanipulated SNF_1 mice (Fig. 4). To assess whether this was due to deletion of autoimmune B cells or due to anergy, purified (T cell-depleted) B cells from the treated mice were stimulated with anti-Ig F(ab′)$_2$ in the presence or absence of rIL-4, and the production of IgG anti-dsDNA, ssDNA, nucleosome, and histones were measured. Saline-treated or $OVA_{323-339}$ peptide-treated mice produced high levels of autoantibodies, whereas B cells from the $H4_{16-39}$ peptide-treated group did not respond even when stimulated with anti-Ig F(ab′)$_2$ in the presence of high levels of rIL-4, suggesting a possible deletion of autoimmune B cells (Fig. 5). In contrast, B cells from $H4_{71-94}$ peptide-treated mice could be stimulated to produce autoantibodies to levels similar to the saline-injected mice (data not shown). The

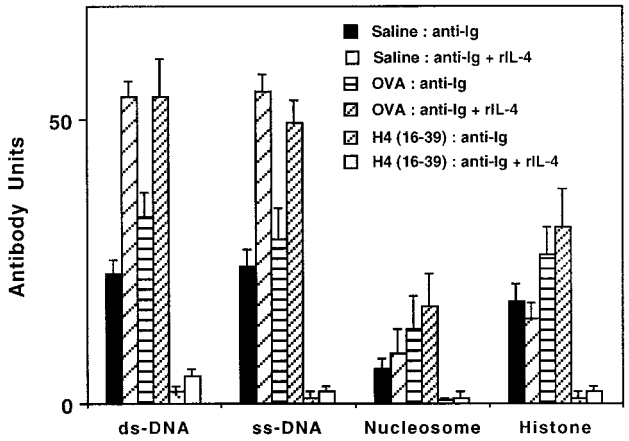

FIGURE 5. Effect of $H4_{16-39}$ peptide treatment on autoimmune B cells. Purified B cells from $H4_{16-39}$, $OVA_{323-339}$, or saline-treated mice (treated as in Fig. 2) were cultured for 7 days in the presence of 10 μg/ml of anti-Ig $F(ab')_2$ (in the absence of any T cells). rIL-4 (50 U/ml) was added at the start of the 7-day culture period in some wells. Units of autoantibody production are expressed in mean ± SEM/dl from five experiments. In this figure and in Figs. 6 and 7, the baseline autoantibody levels produced by B cells cultured alone were: anti-dsDNA, 2.1 ± 0.72; anti-ssDNA, 1.9 ± 0.1; anti-nucleosome, 1.9 ± 1.0; and anti-histone, 2.0 ± 1.6.

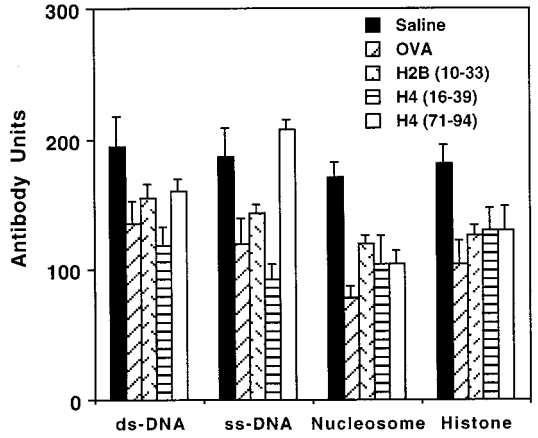

FIGURE 7. B cell response to LPS in peptide-treated mice. B cells (T cell-depleted) were isolated from the different peptide-treated mice or the control saline-injected mice and stimulated with LPS (10 μg/ml), and the IgG autoantibody levels were measured after 7 days of culture. The results are expressed as mean ± SEM U/dl from five experiments. The baseline autoantibody levels produced by B cells cultured alone were same as in Fig. 5.

basal level of autoantibody production by B cells cultured alone ranged from 1.9 to 2.1 U (Fig. 5).

The effect of soluble CD40L and rIL-4 on B cells of $H4_{16-39}$ peptide-treated mice

Soluble CD40L-CD8 fusion protein was added to the B cell cultures at a 1:4 final concentration with or without rIL-4. Purified (T cell-depleted) B cells from the saline-treated or $OVA_{323-339}$ peptide-treated mice produced anti-nuclear autoantibodies upon stimulation with CD40L (Fig. 6). The addition of rIL-4 to these cultures enhanced the production of Abs by B cells from the saline-treated mice further: anti-dsDNA increased from 22 ± 3.2 to 60 ± 4.1 U, anti-ssDNA from 25 ± 3.5 to 54 ± 2.2 U, anti-nucleosome from 7 ± 1.8 to 75 ± 5.6 U, and anti-histone from 19 ± 2.7 to 70 ± 3.3 U. However, the levels of augmentation with soluble CD40L and IL-4 were much lower than that induced by intact Th cells from the autoimmune mice (Fig. 4 vs Fig. 6), indicating that additional molecules/mechanisms might be involved. B cells from the $H4_{16-39}$ peptide-treated group did not produce significant amounts of autoantibodies even with CD40L and rIL-4. (Fig. 6).

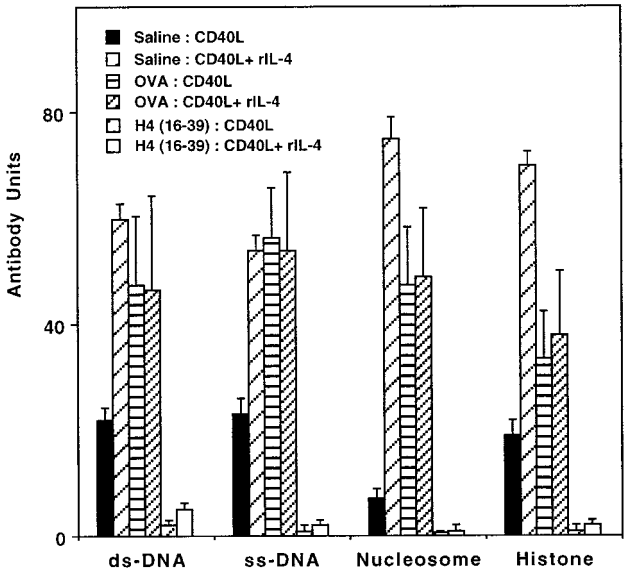

FIGURE 6. Effect of soluble CD40L and IL-4 on B cells from $H4_{16-39}$ or $OVA_{323-339}$ peptide-treated mice. Purified (T cell-depleted) B cells from saline-treated or $H4_{16-39}$ or $OVA_{323-339}$ peptide-treated mice were stimulated with soluble CD40L-CD8 fusion protein, with or without rIL-4 (50 U/ml). After 7 days, the culture supernatants were assayed for autoantibody levels. The results are expressed in mean units ± SEM/dl from five experiments.

The B cell response to LPS in peptide-treated mice

Purified B cells (T cell-depleted) were isolated from the peptide-treated mice and stimulated with the potent mitogen LPS. B cells from all the groups responded by augmenting autoantibody production to levels comparable to that of the saline-treated group (p = 0.07–0.1; Fig. 7).

IgG anti-nuclear autoantibody levels in sera of peptide-treated mice

Sera were collected from the mice at the start of treatment, at 36 wk of age (a time point of greatest difference in incidence of severe nephritis between peptide-treated vs control-saline group; Fig. 1), and at the peak of the disease. IgG Ab levels were measured for anti-dsDNA, ssDNA, nucleosome, and histone at a 1:400 dilution of sera. The anti-dsDNA Ab level in the saline-treated group (mean ± SEM) was 5.5 ± 0.3 U, and in the $OVA_{323-339}$, $H2B_{10-33}$, $H4_{16-39}$, and $H4_{71-94}$ groups these levels were 8.0 ± 1.0, 7.7 ± 0.9, 9.0 ± 2.1, and 5.4 ± 1.1 U, respectively, at the start of treatment. At 36 wk of age in the saline-injected group, anti-dsDNA levels went up to 26 ± 3.2 U, but were 15.6 ± 4.2, 14.0 ± 3.2, 13.0 ± 4.0, and 17.1 ± 2.7 U in the $OVA_{323-339}$, $H2B_{10-33}$, $H4_{16-39}$, and $H4_{71-94}$ groups, respectively (compared with the saline-injected group, the p values ranged from 0.03 to 0.05) (Fig. 8). Similar time point comparisons of anti-ssDNA and anti-nucleosome Ab levels also showed a reduction in the peptide-treated mice that was comparable to the reduction in their anti-dsDNA autoantibody levels (p = 0.04–0.05). The levels of anti-histone Abs varied in different groups (p = 0.3–0.01; Fig. 8). At the time of sacrifice, when the mice had developed severe nephritis

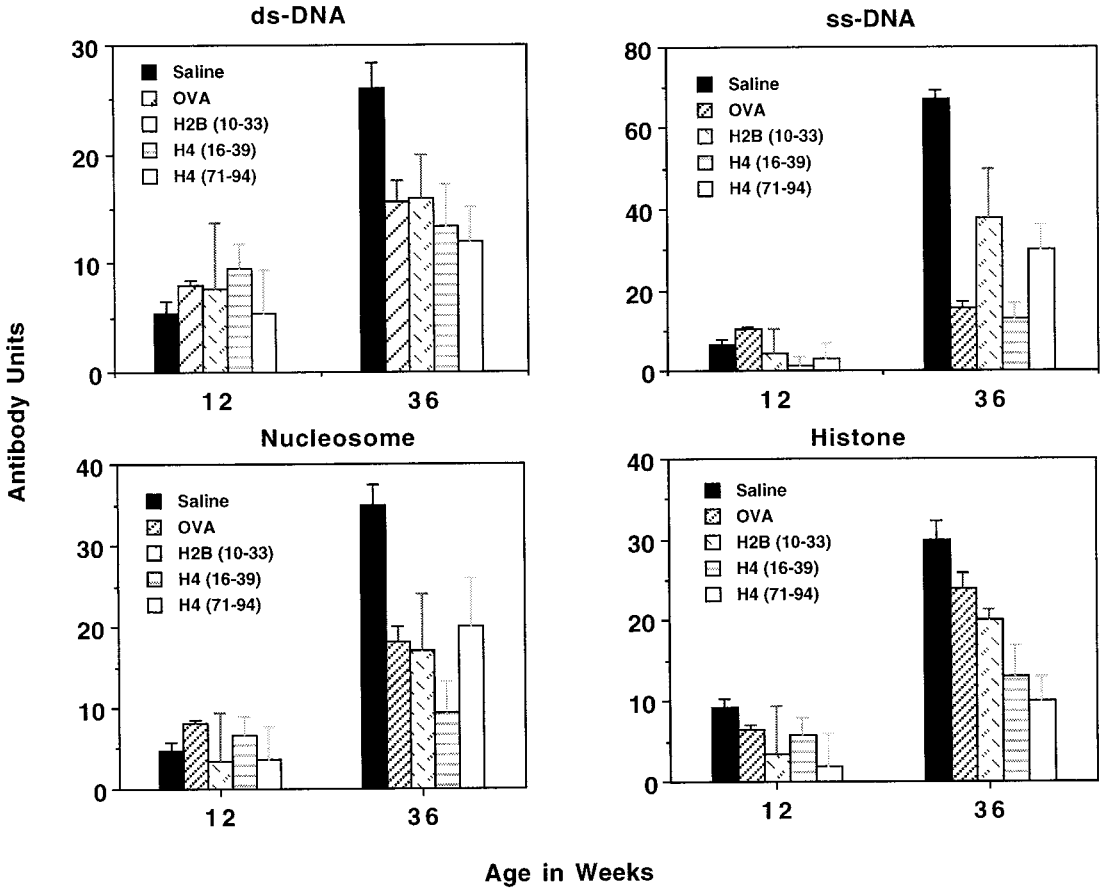

FIGURE 8. IgG autoantibody levels in the serum of peptide-treated mice. SNF_1 mice were bled at the start of the brief therapy with peptides (Fig. 1) and at 36 wk of age (widest difference between control and treated mice in disease incidence) and were assayed for IgG autoantibody levels. The results are expressed in mean ± SEM U/dl.

(Fig. 1), the serum levels of autoantibodies were similar among the peptide- and saline-treated groups (data not shown). Total polyclonal IgG levels were not significantly different in the peptide-treated group from the saline-control group, varying from 8–11 mg/ml.

The search for T cells that could down-regulate autoantibody production in peptide-treated mice

To find if any regulatory T cells might have been generated by peptide therapy, the ability of the T cells from treated mice to inhibit the autoantibody production in cocultures of T and B cells from unmanipulated SNF_1 mice was determined. T cells or purified $CD4^+$ or $CD8^+$ subsets of T cells from the short-term follow-up batch of tolerized or control mice were cocultured with splenic B and T cell mixtures from unmanipulated SNF_1 mice in 24-well plates for 7 days. IgG Abs against ssDNA, dsDNA, histones, and nucleosomes were estimated. No significant reduction in autoantibody production by the addition of T cells from the peptide-treated mice was observed (data not shown).

Treatment of established glomerulonephritis with nucleosomal peptides

Among unmanipulated SNF_1 mice, a small fraction of animals develop severe renal disease relatively later than others. We followed >1000 animals and found 30 18-mo-old SNF_1 mice that had established glomerulonephritis with persistent proteinuria of 300 mg/dl. These old mice (six/group) were chronically treated every month with the nucleosomal histone peptides $H4_{16-39}$, $H4_{71-94}$, or $H2B_{10-33}$ or the $OVA_{323-339}$ peptide (300 μg/mouse). The mice were monitored by measuring proteinuria until they died. The saline-injected mice showed rapid progression of disease and died within 2 mo after the start of the treatment. At this time point, 66.6% of the $H4_{71-94}$ and $H2B_{10-33}$ peptide-injected group of animals were alive ($p = 0.061$), whereas none of the animals treated with $OVA_{323-339}$, or $H4_{16-39}$ had died ($p = 0.002$), even at 22 mo of age (Fig. 9). All of the peptide-treated groups maintained their starting levels of proteinuria during the course of the therapy, with the exception of the $H4_{16-39}$ peptide-injected group, where 66.6% of the animals actually showed a reduction in proteinuria levels from 300-1000 mg/dl to <100 mg/dl. By 26 mo of age, all $H4_{16-39}$ peptide-treated mice remained alive; in contrast, all $H4_{71-94}$ peptide-treated mice were dead ($p = 0.002$), and only one animal in the $OVA_{323-339}$ and $H2B_{10-33}$ peptide-treated groups survived ($p = 0.015$ vs $H4_{16-39}$ group; Fig. 9).

Discussion

These studies show that despite an intrinsic polyclonal hyperactivity and a lowering of the threshold of activation (9, 10, 12–17), pathogenic T and B cells of established lupus can still be functionally down-regulated with tolerogenic administration of appropriate autoepitopes. The results also highlight the importance of nucleosomes as a dominant player in the pathogenic autoimmune response that evolves from or with the polyclonal hyperactivity (1, 5, 23, 29–34). Nucleosomes are natural products of apoptosis (35), and the potential T and B cell repertoire for nucleosomal autoantigens is vast (4, 36). However, the normal immune system ignores

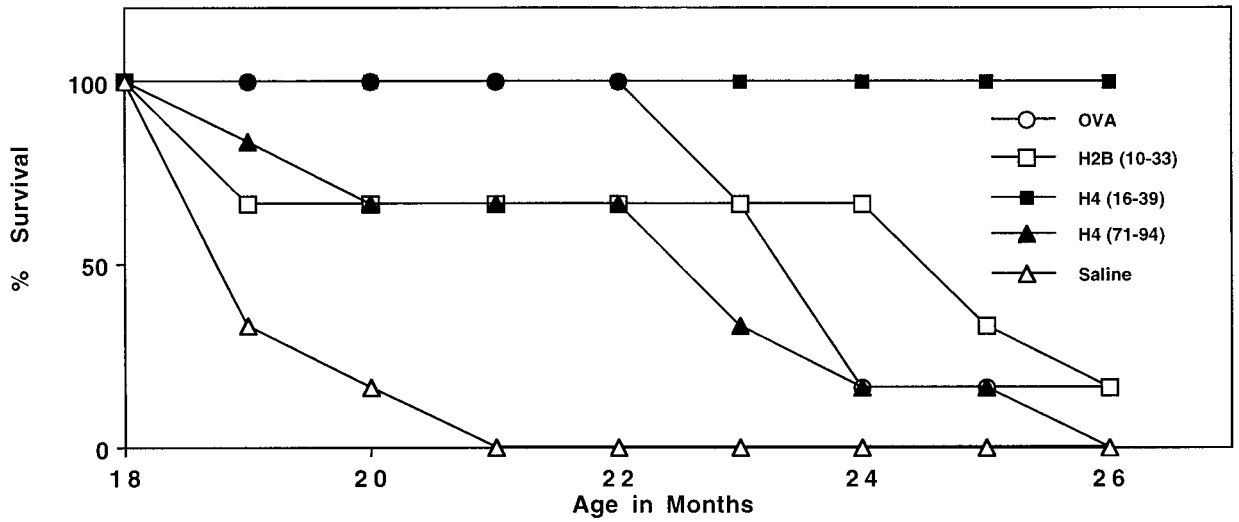

FIGURE 9. Treatment of established lupus nephritis with nucleosomal peptides. Eighteen-month-old SNF_1 mice (six/group) with established glomerulonephritis were injected with 300 μg/mouse of respective peptides per month. The animals were followed by proteinuria measurements until they died. The mice that were alive continued to receive the peptide injections every month. The results are expressed as the percentage of animals that survived.

such products of apoptosis under physiological conditions (37–39). The spontaneous emergence of T cells primed to nucleosomal Ags early in life is a lupus-specific event that is genetically programmed (1, 40).

The mechanism of the "tolerogenic" effect of the nucleosomal peptides on the diminishing autoantibody-inducing ability of the Th cells of lupus is unknown. Anergy of the autoimmune Th cells in the conventional sense is unlikely, because their helper activity in autoantibody production could not be revived by supplements of IL-2 (Fig. 3) or stimulation with nucleosomal peptides plus IL-2 (data not shown). Moreover, we did not detect any immune deviation in cytokine profiles of T cells from the treated mice. The administration of high doses of soluble protein or peptide Ags is known to cause tolerance by apoptosis or anergy of cognate T cells or by generation of regulatory T cells (reviewed in Ref. 8). Some deletion of autoimmune Th cells in the treated mice here remains a possibility, but it is probably minor, because cytokine responses to the peptides were not different between T cells from the saline control and the peptide-treated groups. Perhaps the autoantigen-primed memory T cells of lupus are more resistant to deletion or anergy by these criteria. Moreover, no evidence of down-regulatory T cells were detected in the treated mice. Therefore, the peptide therapy might have impaired some unknown signals or mechanisms involved in the interactions between autoimmune T and B cells that are required for the specialized function of pathogenic autoantibody production. Indeed, this special functional ability to induce pathogenic autoantibodies may require some maturational event(s), because it is detectable in T cells of older, 3- to 4-mo-old SNF_1 mice, whereas the T cells of younger mice are incapable of providing such help (26), although they respond to nucleosomes spontaneously and hyperexpress CD40L (1, 14). The superior autoantibody-inducing ability of intact T cells, as compared with soluble CD40L and IL-4 (Fig. 4 vs Fig. 6), also suggests the involvement of additional mechanisms. Furthermore, what is striking here is that any one of the nucleosoamal peptide autoepitopes we identified could diminish pathogenic autoantibody production across the board (tolerance spreading) and markedly delay the development of nephritis.

Large doses of soluble peptides could competitively block immunogenic presentation of autoepitopes by displacing them from class II molecules of APC and also by being predominently displayed on class II molecules of resting APC (41–44). In fact, the former may be the reason for the beneficial effect of the OVA peptide, which binds strongly to $I\text{-}A^d$ (21, 41, 42). Alternatively, the OVA peptide, which also bears charged residues, could have acted as an antagonist or an altered peptide ligand for the promiscuous T cells of lupus (4, 21, 41, 42). But, unlike the nucleosomal peptides, immunization with the OVA peptide in CFA does not induce lupus nephritis or stimulate the pathogenic Th cells of lupus (3). Tolerization with peptides corresponding to complementarity-determining regions 1 and 2 of the V_H region of an anti-DNA autoantibody that is recurrently expressed in SNF_1 mice (1), or with the complementarity-determining region peptide of a nonspecific control, anti-malaria Ab (1), does not have any significant effect on the incidence of nephritis in the SNF_1 mice (S. Adams, P. LeBlanc, and S.K. Datta, unpublished observations). However, in the $(NZB \times NZW)F_1$ mice, tolerization with autoantibody-derived peptides does have a therapeutic effect (45), and interestingly such peptides have charged residues like the nucleosomal peptides. In the future, studies to define the TCR and MHC contact residues in the nucleosomal peptide autoepitopes may reveal the mechanism of the beneficial effect of the OVA peptide.

The second reason for "tolerance spreading" could be because individual TCRs of the pathogenic autoantibody-inducing Th cells of lupus recognize more than one nucleosomal peptide epitope in a promiscuous or degenerate fashion and in the context of diverse class II molecules (3, 4). High-affinity interactions between the lupus TCRs and MHC-nucleosomal peptide complex due to reciprocally charged residues probably overcomes the requirement for MHC restriction. Therefore, a single nucleosomal epitope with charged residues could possibly tolerize a spectrum of lupus Th cells that could recognize, in different registers, one or two shared residues in apparently different peptide autoepitopes. This plasticity of TCRs is being increasingly appreciated by structural and functional analysis (4, 46–51).

The third possibility for "tolerance spreading" is the multipotent and promiscuous helper activity of the pathogenic Th cells of lupus. Remarkably, a single lupus Th clone can help either a dsDNA-specific, ssDNA-specific, histone-specific, high-mobility group chromosomal protein-specific, or nucleosome-specific B cell, because each of these B cells by binding to its respective epitope on the whole chromatin can take it up and process and then

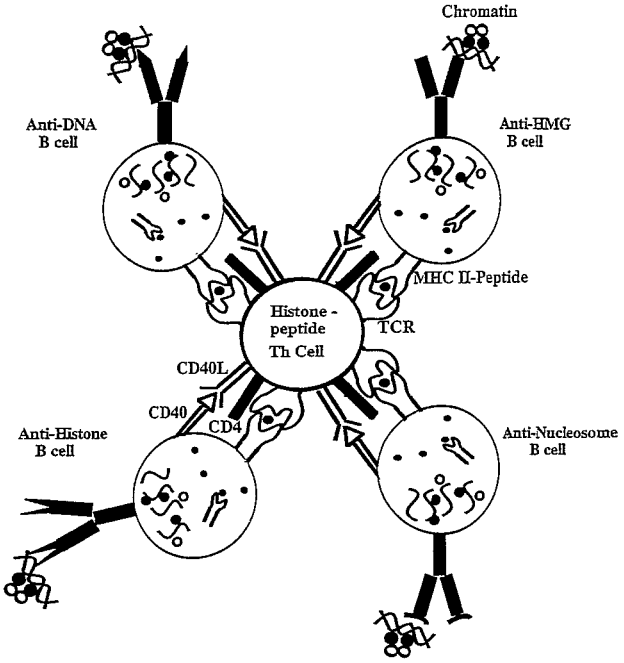

FIGURE 10. Production of diverse autoantibodies in SLE by promiscuous help. In this instance, a single Th cell that is specific for a nucleosomal histone peptide could help different autoimmune B cells that are either specific for DNA, nucleosomes, histones, or the nonhistone high-mobility group chromosomal protein (1, 2). Each of these types of B cells would bind to its respective epitope in chromatin, but take up and process the whole nucleosome particle and then present the relevant peptide epitope in the core histone to this particular Th clone (3). This principle of multipotent, intermolecular help involving different B cell epitopes in the complex chromatin particle would also apply to other pathogenic autoantibody-inducing Th cells of lupus that are specific for high-mobility group chromosomal protein or other nucleosomal core histone-peptides (reproduced with permission Ref. 52).

present the relevant peptide epitope in the chromatin to the Th clone (Fig. 10, and Refs. 1, 2, and 52), resulting in intermolecular help. Therefore, the multipotent (promiscuous helper) Th cells of lupus cause immediate epitope diversification, rather than the sequential epitope spreading that comes with inflammatory damage and the progression of autoimmune disease (24, 53, 54). Tolerization of such Th cells would obviously deprive multiple autoimmune B cells of T cell help.

Among the three nucleosomal peptides, $H4_{16-39}$ showed the most beneficial effect. Interestingly, the $H4_{16-39}$ peptide is not the most immunogenic among the nucleosomal autoepitopes in triggering pathogenic Th cells when administered with CFA into SNF_1 mice (3), nor does it have the highest affinity for MHC class II molcules (4). But, $H4_{16-39}$, administered i.v., was able to tolerize both the autoimmune Th cells and the B cells of lupus. Autoimmune memory B cells were probably most affected by this peptide tolerogen, as they could not be rescued by CD40L plus IL-4 or anti-Ig plus IL-4 stimulation. The addition of autoimmune Th cells from unmanipulated SNF_1 mice did increase autoantibody production by B cells from the $H4_{16-39}$ peptide-treated animals above baseline but not anywhere comparable to the other groups. Apoptosis of mature B cells with high doses of peptide i.v. has been observed in the lysozyme system (55), and it could have contributed to the tolerogenic effect of $H4_{16-39}$ peptide. Stimulation with the highly potent mitogen LPS did bring back autoantibody production to some extent in the B cells of $H4_{16-39}$ peptide-tolerized mice, probably by stimulating severely anergized or other subsets of naive but potentially autoimmune B cells. Anergic B cells could be tolerogenic to autoimmune T cells because they would present nucleosomal autoantigens without providing costimulation (12, 14, 15, 43, 56, 57). B cell epitope mapping of histones targeted by autoantibodies found in spontaneous SLE is still evolving from earlier studies (31, 58, 59). Recent studies indicate not all anti-histone reactivities are equal. Anti-histone Abs that react exclusively with histones are probably not pathogenic in lupus, but anti-nucleosome Abs are (1, 29, 30, 33, 34, 58, 60). The latter pathogenic autoantibodies see different epitopes, including exposed as well as buried ones in core histones of nucleosomes, and conformational determinants are yet to be identified (29, 61). Nevertheless, $H4_{16-39}$ falls within the region targeted by lupus autoantibodies. As shown here, the overlapping of epitopes for pathogenic Th cells and autoimmune B cells of lupus makes $H4_{16-39}$ a highly efficient tolerogen, and this principle might be relevant to other autoimmune diseases as well (62, 63). We have previously identified an additional epitope in nucleosomal core histone H3, $H3_{85-102}$, and the splenic T cells of prenephritic SNF_1 mice spontaneously responded to this peptide (3). Interestingly, this T cell epitope is also bound by spontaneously arising anti-DNA autoantibodies of lupus (64). Future studies should determine whether $H3_{85-102}$ is also a potent tolerogen for therapy of lupus nephritis in SNF_1 mice. Thus, autoantigen-experienced and presumably memory T and B cells of lupus can be functionally inactivated, at least for their ability to produce pathogenic autoantibodies by tolerogenic therapy with nucleosomal peptides. Finally, despite tolerance spreading, the peptide-treated mice did not develop any generalized immunosuppression, they were housed in conventional cages, and their total serum IgG levels were not affected by the therapy.

References

1. Mohan, C., S. Adams, V. Stanik, and S. K. Datta. 1993. Nucleosome: a major immunogen for the pathogenic autoantibody-inducing T cells of lupus. *J. Exp. Med. 177:1367.*
2. Desai-Mehta, A., C. Mao, S. Rajagopalan, T. Robinson, and S. K. Datta. 1995. Structure and specificity of T-cell receptors expressed by pathogenic anti-DNA autoantibody-inducing T cells in human lupus. *J. Clin. Invest. 95:531.*
3. Kaliyaperumal, A., C. Mohan, W. Wu, and S. K. Datta. 1996. Nucleosomal peptide epitopes for nephritis-inducing T helper cells of murine lupus. *J. Exp. Med. 183:2459.*
4. Shi, Y., A. Kaliyaperumal, L. Lu, S. Southwood, A. Sette, M. A. Michaels, and S. K. Datta. 1998. Promiscuous presentation and recognition of nucleosomal autoepitopes in lupus: role of autoimmune T cell receptor α chain. *J. Exp. Med. 187:367.*
5. Voll, R. E., E. A. Roth, I. Girkontaite, H. Fehr, M. Herrmann, H. M. Lorenz, and J. R. Kalden. 1997. Histone-specific Th0 and Th1 clones derived from systemic lupus erythematosus patients induce double-stranded DNA antibody production. *Arthritis. Rheum. 40:2162.*
6. Kretz-Rommel, A., S. R. Duncan, and R. L. Rubin. 1997. Autoimmunity caused by disruption of central T cell tolerance: a murine model of drug induced lupus. *J. Clin. Invest. 99:1888.*
7. Nakajima, A., S. Hiroshe, H. Yagita, and K. Okomura. 1997. Roles of IL-4 and IL-12 in the development of lupus in NZB/W F1 mice. *J. Immunol. 158:1466.*
8. Liblau, R., R. Tisch, N. Bercovici, and H. O. McDevitt. 1997. Systemic antigen in the treatment of T-cell mediated autoimmune diseases. *Immunol. Today 18: 599.*
9. Datta, S. K., F. L. Owen, J. E. Womack, and R. J. Riblet. 1982. Analysis of recombinant inbred lines derived from autoimmune (NZB) and high leukemia (C58) strains: independent multigenic systems control B cell hyperactivity, retrovirus expression and autoimmunity. *J. Immunol. 129:1539.*
10. Klinman, D. M., and A. D. Steinberg. 1987. Systemic autoimmune disease arises from polyclonal B cell activation. *J. Exp. Med. 165:1755.*
11. Cohen, P. L., and R. A. Eisenberg. 1991. lpr and gld: single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu. Rev. Immunol. 9:243.*
12. Chan, O., and M. J. Shlomchik. 1998. A new role for B cells in systemic autoimmunity: B cells promote spontaneous T cell activation in MRL-lpr/lpr mice. *J. Immunol. 160:51.*
13. Jongstra-Bilen, J., B. Vukusic, K. Boras, and J. E. Wither. 1997. Resting B cells from autoimmune lupus-prone NZB and (NZB $\times$ NZW)F_1 mice are hyper-responsive to T cell-derived stimuli. *J. Immunol. 159:5810.*

14. Mohan, C., Y. Shi, J. D. Laman, and S. K. Datta. 1995. Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. *J. Immunol. 154:1470.*
15. Desai-Mehta, A., L. Lu, R. Ramsey-Goldman, and S. K. Datta. 1996. Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. *J. Clin. Invest. 97:2063.*
16. Koshy, M., D. Berger, and M. K. Crow. 1996. Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. *J. Clin. Invest. 98:826.*
17. Liossis, S.-N., B. Kovacs, G. Dennis, G. M. Kammer, and G. C. Tsokos. 1996. B cells from patients with systemic lupus erythematosus display abnormal antigen receptor-mediated early signal transduction events. *J. Clin. Invest. 98:2549.*
18. Wakeland, E. K., L. Morel, C. Mohan, and M. Yui. 1997. Genetic dissection of lupus nephritis in murine models of SLE. *J. Clin. Immunol. 17:272.*
19. Vyse, T. J., and B. L. Kotzin. 1996. Genetic basis of systemic lupus erythematosus. *Curr. Opin. Immunol. 8:843.*
20. Kono, D. H., and A. N. Theofilopoulos. 1996. Genetic contributions to SLE. *J. Autoimmunity 9:437.*
21. Sette, A., S. Buus, C. Colon, C. Miles, and H. M. Gray. 1988. I-A^d binding peptides derived from unrelated protein antigens share a common structural motif. *J. Immunol. 141:45.*
22. Eastcott, J. W., R. S. Schwartz, and S. K. Datta. 1983. Genetic analysis of the inheritence of B cell hyperactivity in relation to the development of autoantibodies and glomerulonephritis in NZB $\times$ SWR crosses. *J. Immunol. 131:2232.*
23. Adams, S., P. LeBlanc, and S. K. Datta. 1991. Junctional region sequences of T-cell receptor β chain genes expressed by pathogenic anti-DNA autoantibody-inducing T helper cells from lupus mice: possible selection by cationic autoantigens. *Proc. Natl. Acad. Sci. USA 88:11271.*
24. Kalled, S. L., A. H. Cuttler, S. K. Datta, and D. W. Thomas. 1998. Anti-CD40 ligand antibody treatment of SNF_1 mice with established nephritis: preservation of kidney function. *J. Immunol. 160:2158.*
25. Gaynor, B., C. Putterman, P. Valadon, L. Spatz, M. D. Scharff, and B. Diamond. 1997. Peptide inhibition of glomerular deposition of an anti-DNA antibody. *Proc. Natl. Acad. Sci. USA 94:1955.*
26. Datta, S. K., H. Patel, and D. Berry. 1987. Induction of a cationic shift in IgG anti-DNA autoantibodies: role of T helper cells with classical and novel phenotypes in three murine models of lupus nephritis. *J. Exp. Med. 165:1252.*
27. Sainis, K., and S. K. Datta. 1988. $CD4^+$ T cell lines with selective patterns of autoreactivity as well as $CD4^-/CD8^-$ T helper cell lines augment the production of idiotypes shared by pathogenic anti-DNA autoantibodies in the NZB $\times$ SWR model of lupus nephritis. *J. Immunol. 140:2215.*
28. Wortis, H. H., M. Teutsch, M. Higer, J. Zheng, and D. C. Parker. 1995. B-cell activation by crosslinking of surface IgM or ligation of CD40 involves alternative signal pathways and results in different B cell phenotypes. *Proc. Natl. Acad. Sci. USA 92:3348.*
29. Berden, J. H. 1997. Lupus nephritis. *Kidney Int. 52:538.*
30. Burlingame, R. W., R. L. Rubin, R. S. Balderas, and A. N. Theofilopoulos. 1993. Genesis and evolution of anti-chromatin autoantibodies in murine lupus implicates immunization with self antigen. *J. Clin. Invest. 91:1687.*
31. Hardin, J. A., and J. O. Thomas. 1983. Antibodies to histones in systemic lupus erythematosus: localization of prominent autoantigens on histone H1 and H2B. *Proc. Natl. Acad. Sci. USA 80:7410.*
32. Tan, E. M. 1989. Anti-nuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol. 44:93.*
33. Amoura, Z., H. Chabre, S. Koutouzov, C. Lotton, A. Cabrespines, J. F. Bach, and L. Jacob. 1994. Nucleosome-restricted antibodies are detected before anti-dsDNA and anti-histone antibodies in serum of MRL-Mp lpr/lpr and +/+ mice, and are present in kidney eluates of lupus mice with proteinuria. *Arthritis Rheum. 37:1684.*
34. Lefkowith, J. B., and G. S. Gilkeson. 1996. Nephritogenic autoantibodies in lupus: current concepts and continuing controversies. *Arthritis Rheum. 39:894.*
35. Casciola-Rosen, L. A., G. Anhalt, and A. Rosen. 1994. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med. 179:1317.*
36. Stewart, A., C. Huang, A. Long, B. Stollar, and R. Schwartz. 1992. VH-gene representation in autoanitbodies reflects the normal B cell repertoire. *Immunol. Rev. 128:101.*
37. Voll, R. E., M. Herrmann, E. A. Roth, C. Stach, J. R. Kalden, and I. Girkontaite. 1997. Immunosuppressive effects of apoptotic cells. *Nature 390:350.*
38. Fadok, V. A., D. L. Bratton, A. Konowal, P. W. Freed, J. Y. Westcott, and P. M. Henson. 1998. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE_2, and PAF. *J. Clin. Invest. 101:890.*
39. Mevorach, D., J. L. Zhou, X. Song, and K. B. Elkon. 1998. Systemic exposure to irradiated apoptotic cells induces autoanitbody production. *J. Exp. Med. 188:387.*
40. Mohan, C., E. Alas, L. Morel, Y. Ping, and E. K. Wakeland. 1998. Genetic dissection of SLE pathogenesis: *Sle*1 on murine chromosome 1 leads to a selective loss of tolerance to H2A/H2B/DNA subnucleosome. *J. Clin. Invest. 101:1362.*
41. Lamont, A. G., A. Sette, R. Fujinami, S. M. Colon, C. Miles, and H. M. Grey. 1990. Inhibition of experimental autoimmune encephalomyelitis induction in SJL/J mice by using a peptide with high affinity for I-A^s molecules. *J. Immunol. 145:1687.*
42. Kuchroo, V. K., J. M. Greer, D. Kaul, G. Ishioka, A. Franco, A. Sette, R. A. Sobel, and M. B. Lees. 1994. A single TCR antagonist peptide inhibits experimental allergic encephalomyelitis mediated by a diverse TCR repertoire. *J. Immunol. 153:3326.*
43. Eynon, E. E., and D. C. Parker. 1992. Small B cells as antigen presenting cells in the induction of tolerance to soluble protein antigens. *J. Exp. Med. 175:131.*
44. Zhong, G., C. R. Sousa, and R. N. Germain. 1997. Antigen-unspecific B cells and lymphoid dendritic cells both show extensive surface expression of processed antigen-MHC class II complexes after soluble protein exposure in vivo or in vitro. *J. Exp. Med. 186:673.*
45. Singh, R. R., F. M. Ebling, E. E. Sercarz, and B. H. Hahn. 1995. Immune tolerance to autoantibody-derived peptides delays development of autoimmunity in murine lupus. *J. Clin. Invest. 96:2990.*
46. Evavold, B. D., J. Sloan-Lancaster, K. J. Wilson, J. B. Rothbard, and P. M. Allen. 1995. Specific T cell recognition of minimally homologous peptides: evidence for multiple endogenous ligands. *Immunity 2:655.*
47. Wucherpfennig, K., and J. L. Strominger. 1995. Molecular mimicry in T cell mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell 80:695.*
48. Bhardwaj, V., V. Kumar, H. M. Geysen, and E. E. Sercarz. 1993. Degenerate recognition of a dissimilar antigenic peptide by myelin basic protein-reactive T cells. *J. Immunol. 151:5000.*
49. Hemmer, B., M. Vergelli, B. Gran, N. Ling, P. Conlon, C. Pinilla, R. Houghten, H. F. McFarland, and R. Martin. 1998. Predictable TCR antigen recognition based on peptide scans leads to the identification of agonist ligands with no sequence homology. *J. Immunol. 160:3631.*
50. Mason, D. 1998. A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol. Today 19:395.*
51. Garcia, K. C., M. Degano, L. R. Pease, M. Huang, P. A. Peterson, L. Teyton, and I. A. Wilson. 1998. Structural basis of plasticity in T cell receptor recognition of a self peptide-MHC antigen. *Science 279:1166.*
52. Datta, S. K., and A. Kaliyaperumal. 1997. Nucleosome-driven autoimmune response in lupus: pathogenic T helper cell epitopes and costimulatory signals. *Ann. NY Acad. Sci. 815:155.*
53. Lehman, P., T. Forsthuber, A. Miller, and E. Sercarz. 1992. Spreading of T cell autoimmunity to cryptic determinants of an autoantigen. *Nature 358:155.*
54. Craft, J., and S. Fatenejad. 1997. Self antigens and epitope spreading in systemic autoimmunity. *Arthritis Rheum. 40:1374.*
55. Shokat, K. M., and C. C. Goodnow. 1995. Antigen-induced B-cell death and elimination during germinal center immune responses. *Nature 375:334.*
56. Mamula, M. J., S. Fatenejad, and J. Craft. 1994. B cells process and present lupus autoantigens that initiate autoimmune T cell responses. *J. Immunol. 152:1453.*
57. Buhlmann, J. E., T. M. Foy, A. Aruffo, K. M. Crassi, J. A. Ledbetter, W. R. Green, J. C. Xu, L. D. Shultz, D. Roopesian, R. A. Flavell, L. Fast, R. J. Noelle, and F. H. Durie. 1995. In the absence of a CD40 signal B cells are tolerogenic. *Immunity 2:645.*
58. Monestier, M., and B. L. Kotzin. 1992. Antibodies to histones in systemic lupus erythematosus and drug-induced lupus syndromes. *Rheum. Dis. Clin. North Am. 18:415.*
59. Gohill, J., P. D. Cary, M. Couppez, and M. J. Fritzler. 1985. Antibodies from patients with drug-induced and idiopathic lupus erythematosus react with epitopes restricted to the amino and carboxyl termini of histones. *J. Immunol. 135:3116.*
60. Ohnishi, K., F. M. Ebling, B. Mitchell, R. R. Singh, B. H. Hahn, and B. P. Tsao. 1994. Comparison of pathogenic and non-pathogenic murine antibodies to DNA: antigen binding and structural characteristics. *Int. Immunol. 6:817.*
61. Stemmer, C., J.-P. Briand, and S. Muller. 1997. Mapping of linear histone regions exposed at the surface of the nucleosome in solution. *J. Mol. Biol. 273:52.*
62. Tung, K. S. K., Y. H. Lou, K. M. Garza, and C. Teuscher. 1997. Autoimmune ovarian disease:mechanism of disease induction and prevention. *Curr. Opin. Immunol. 9:839.*
63. Wucherpfennig, K. W., I. Catz, S. Hausman, J. L. Strominger, L. Steinman, and K. G. Warren. 1997. Recognition of immunodominant myelin basic protein peptide by autoantibodies and HLA-DR2 restricted T cell clones from multiple sclerosis patients. *J. Clin. Invest. 100:1114.*
64. Stemmer, C., P. Richalet-Secordel, M. C. J. Van Bruggen, C. Kramers, J. H. M. Berden, and S. Muller. 1996. Dual reactivity of several monoclonal anti-nucleosome antibodies for double stranded DNA and a short segment of histone H3. *J. Biol. Chem. 271:21257.*