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*J Immunol* 1999; 162:5775-5783; 
http://www.jimmunol.org/content/162/10/5775

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

Arunan Kaliyaperumal, Marissa A. Michaels, and Syamal K. Datta

In the (SWR × NZB)F₁ 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)F₁ 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)F₁ 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 Th 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 H4₁₆₋₃₉, 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)F₁ (SNF₁) 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 SNF₁ mice are spontaneously primed from early life to these disease-relevant epitopes (3). Moreover, immunization of preautoimmune SNF₁ mice with these nucleosomal autoantigenic epitopes 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 autoimmune 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, SNF₁ mice could delay the development of lupus nephritis. Moreover, chronic tolerogenic therapy with the peptides administered into much older SNF₁ mice with established glomerulonephritis prolonged survival and even checked the progression of disease. Remarkably, the best therapeutic effect was obtained with the peptide H4₁₆₋₃₉, which had a tolerogenic effect on both autoimmune Th and B cells.

Materials and Methods

Mice

NZB and SWR mice were purchased from Jackson Laboratory (Bar Harbor, ME). SNF₁ 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 H4₁₆₋₃₉, H4₇₁₋₉₄, and H2B₁₀₋₃₉ (3). We also used an I-A^d binding, 17-mer OVA (OVA₁₂₃₋₃₃₉) peptide that does not accelerate disease in SNF₁ 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 SNF₁ females that were 12-wk-old (nine mice per group), were injected i.v. with either H2B₁₀₋₃₉, H4₁₆₋₃₉, H4₇₁₋₉₄, or OVA₁₂₃₋₃₃₉ 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).
The mice were monitored weekly for proteinuria using albusitx (VWR Scientific, Chicago, IL) and killed when they developed persistent proteinuria (two consecutive weekly readings of 300 mg/dl or greater). Serum were collected for the determination of IgG anti-nuclear autoantibodies. Blood urea nitrogen was measured by azotix (Miles, Ekhart, IN). Kidney sections were stained with hematoxylin and cosin 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 SNF1 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 SNF1 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 SNF1 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 SNF1 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 × 10^6/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 SNF1 mice were cocultured, respectively, with unmanipulated or tolerized SNF1 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, SNF1 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 SNF1 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 SNF1 females that did not have proteinuria (nine mice per group) were injected i.v. with either H2B10–33, H416–39, H471–94, or OVA323–339 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 H471–94 peptide-injected group, and 11.1% (p = 0.131) of the OVA323–339 peptide-injected group of mice developed severe nephritis, whereas the H2B10–33 or H416–39 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 OVA323–339 peptide-injected group of mice developed severe nephritis, whereas the H2B10–33 or H416–39 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 OVA323–339 peptide-injected group of mice developed severe nephritis, whereas the H2B10–33 or H416–39 peptide-injected mice did not develop disease at this time (p = 0.029).
**Table I.** Incidence of lupus nephritis at sacrifice in the short-term batch of mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Grading of Lupus Nephritis (percent incidence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0+</td>
</tr>
<tr>
<td>OVA_{123}</td>
<td>33.3</td>
</tr>
<tr>
<td>H2B_{10-33}</td>
<td>66.6</td>
</tr>
<tr>
<td>H416-39</td>
<td>33.3</td>
</tr>
<tr>
<td>H4_{71-94}</td>
<td></td>
</tr>
</tbody>
</table>

* 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 SNF1 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 [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 SNF1 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 not shown).

**Peptide therapy impairs CD4^{+} T cell help for autoantibody production**

The helper assay for autoantibody-inducing ability is a much more significant 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 SNF1 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_{123}, H4_{16-39}, and H4_{71-94} 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_{123} 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_{123} group the reduction was not significant ($p = 0.061$). The effect on anti-histone Ab induction, overall, was not as dramatic ($p = 0.1$).

**The addition of IL-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 SNF1 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_{123}, 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 antinucleosome 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 SNF1 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...
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 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.

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 SNF1 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 SNF1 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
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).

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...
(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 SNF1 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 SNF1 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 SNF1 mice, a small fraction of animals develop severe renal disease relatively later than others. We followed >1000 animals and found 30 18-mo-old SNF1 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 8. IgG autoantibody levels in the serum of peptide-treated mice. SNF1 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.](http://www.jimmunol.org/)
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 mechanisms involved in the interactions between autoimmune T and B cells that are required for the specialized function of pathogenic autoantibody production. Such Ags early in life is a lupus-specific event that is genetically programmed (1, 40).

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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 SNF1 mice (3), nor does it have the highest affinity for MHC class II molecules (4). But, H4_{16-39} administered i.v. was able to tolerantize 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 SNF1 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 co-stimulation (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_{55-102}, and the splenic T cells of prenephritic SNF1 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_{55-102} is also a potent tolerogen for therapy of lupus nephritis in SNF1 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


