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A Peptide DNA Surrogate Accelerates Autoimmune Manifestations and Nephritis in Lupus-Prone Mice

Erik Beger,* Bisram Deocharan,† Morris Edelman,‡ Bryna Erblich,§ Yun Gu,§ and Chaim Putterman*‡§

Lupus-associated anti-DNA Abs display features of Ag selection, yet the triggering Ag in the disease is unknown. We previously demonstrated that the peptide DWESVWLSN is bound by a pathogenic anti-DNA Ab, and that immunization of nonautoimmune mice with this peptide induces autoantibodies and renal Ig deposition. To elucidate differences in the induced B cell responses in mice genetically predisposed to autoimmunity, young (NZB × NZW)F₁ mice were immunized with this peptide DNA mimotope. DWESVWLSN-immunized mice had significantly increased IgG anti-dsDNA, anti-laminin, and anti-cardiolipin Ab titers compared with controls. In addition, glomerular histopathology in the form of endocapillary disease and crescent formation was markedly more severe in DWESVWLSN-immunized mice. Analysis of mAbs from DWESVWLSN-immunized mice revealed that anti-peptide Abs were often cross-reactive with DNA. Genetic elements used in the Ab response in immunized mice were homologous to those used in the spontaneous anti-DNA response in (NZB × NZW)F₁ mice, as well as in other, experimentally induced anti-DNA Abs. Our results indicate that peptide immunization can induce a molecular genetic response common to a variety of stimuli that break tolerance to mammalian dsDNA. Based on the similarity between spontaneously arising anti-DNA Abs and several types of induced anti-DNA Abs, we suggest that there may be more than a single Ag that can trigger systemic lupus erythematosus.


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3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; FR, framework region; MAP, multiple antigenic peptide; PAS, periodic-acid Schiff.

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1 This work was supported by National Institutes of Health Grant K08 AR02015-02 and the Arthritis Foundation-New York Chapter Robert Wood Johnson Charitable Trust SLE Young Scholar Award (to C.P.).
and 2) the finding that protein Ag alone without DNA is sufficient to induce a pathogenic anti-dsDNA response. Possible mechanisms by which an anti-peptide immune response can lead to the generation of autoantibodies include somatic mutation with generation of novel auto-
tospecificities, B cell Ag presentation of multimolecular nuclear complex-
plexes containing DNA and epitope spreading, and perturbation of the idiotypic network (12).

To address a possible role of peptide Ags in accelerating auto-
immune manifestations and organ damage in genetically predis-
posed mice, and explore differences in B cell responses to the peptide dsDNA mimotope between autoimmune and nonautoim-
mune mice, we immunized (NZB × NZW)F₁ (B/W) mice with the peptide and analyzed the peptide-specific and autoantibody responses.

Materials and Methods

Peptides and immunization

The derivation of DWEYSVWLSN has been described in detail previously
(8). SVIWSWMWL and TIALKLWRWA, 10-mer peptides from the same phage library, were used as controls. Multimeric peptides were pre-
pared for immunization by synthesis on an eight-branched, polylysine backbone (multiple antigenic peptide (MAP); Research Genetics, Huntsville, AL).

B/W mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Immunizations were performed, as previously described (12), with slight modifications. Thirteen-week-old female mice (n = 14) were immu-
низированы с 100 μg of MAP-DWEYSVWLSN emulsified 1:1 in CFA, H37 Ra (Difco, Detroit, MI), followed by a booster of 100 μg/ml in PBS were boiled for 15 min, cooled rapidly on ice, and adsorbed to Immulon II plates at 4°C overnight. PBS was serially diluted on dsDNA-coated plates and the ELISA was continued, as described above. The serum was reported as positive at the highest dilution at which the OD was higher than the mean plus 3 SDs of five control sera (3-mo-old preautoimmune female B/W mice) at that di-
lution, and the titer is given as 1/dilution. For inhibition ELISAs, serum and a dilution resulting in 50% of maximal DNA binding or mAbs at 10
°C for 10 min. PCR products were puri-
ified on a protein G column from

Generation of hybridomas

Thirteen-week-old B/W mice (n = 3; B/W1, B/W2, B/W3) were immu-
nized with 100 μg of MAP-DWEYSVWLSN in CFA on day 0, and boosted twice with 100 μg of MAP-DWEYSVWLSN in IFA. Ten days after the last boost, the mice were sacrificed and the splenocytes were fused to NSO myeloma cells at a 1:1 ratio using established methodology (13, 14). Spleens from two age-matched unmanipulated female B/W mice at 20 wk of age were separately fused as controls. Supernatants from hybrid-
containing wells were screened for binding to peptide and dsDNA by ELISA, as described below. Cells from positive wells were cloned by lim-
iting dilution.

Ab purification

IgM mAbs were purified from hybridoma supernatants on a protein G column from

ELISAs

Salmon sperm dsDNA (Calbiochem Novabiochem, La Jolla, CA) was pu-
рифирована с 0.45-μm filter (Millipore, St. Louis, MO) were adsorbed to Immulon II plates at 4°C overnight. BSA (Roche), cytochrome c, lysozyme, and keyhole limpet he-
mocyanin (Sigma-Aldrich) all at 10 μg/ml in PBS were adsorbed to Im-

Renal histopathology

One kidney from each mouse was obtained at sacri-
fice on wk 16. Control mice received MAP-TIALKWLRWA
(n = 4) or MAP-SVIWSWMWL (n = 5) using the same schedule of adjuvant and immunizations.

Thirteen-week-old B/W mice (n = 14) were immu-
nized with 100 μg of MAP-DWEYSVWLSN in CFA on day 0, and boosted twice with 100 μg of MAP-DWEYSVWLSN in IFA. Ten days after the last boost, the mice were sacrificed and the splenocytes were fused to NSO myeloma cells at a 1:1 ratio using established methodology (13, 14). Spleens from two age-matched unmanipulated female B/W mice at 20 wk of age were separately fused as controls. Supernatants from hybrid-
containing wells were screened for binding to peptide and dsDNA by ELISA, as described below. Cells from positive wells were cloned by lim-
iting dilution.

Ab purification

IgM mAbs were purified from hybridoma supernatants on an immobilized mannan-binding protein column, using the Immunopuro IgM purification kit (Pierce, Rockford, IL), and the manufacturer’s instructions. IgG mAbs were purified from hybridoma supernatants on a protein G column from Amersham Biosciences (Piscataway, NJ).

RNA isolation and PCR of Ig genes

RNA isolation and PCR of Ig genes were performed as previously described (12). Total RNA was extracted from 2 × 10⁵ hybridoma cells, and reverses transcribed using the Superscript Pre-Amplification System (Life Technologies, Gaithersburg, MD), and an oligo(dT) primer. PCR of the κ L chain was performed using a 3′ κ C region primer and a set of seven 5′ degenerate κ L chain primers (15). PCR of the H chain was performed using a set of 10 degenerate 5′ H chain V regions primers, and a 3′ IgC region primer or 3′ universal IgG primer for IgM and the IgG H chain V regions, respectively (15). PCR amplifications were conducted in a PerkinElmer 9700 thermal cycler (PerkinElmer, Palo Alto, CA) for 35 cycles, with a hot start at 94°C for 3 min, denaturation at 94°C for 50 s, annealing at 50°C for 50 s, and extension at 72°C for 40 s, followed by a final extension at 72°C for 10 min. PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA). Ambiguities in the nucleotide sequence were resolved by cloning selected PCR products using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA), and plasmid se-
quencing using the M13 (−20) primer.

Sequence and sequence analysis

Automated sequencing of PCR products was performed at the DNA se-
quencing facility of the Albert Einstein College of Medicine using an Ap-
plied Biosystems 377 sequencer (Applied Biosystems, Foster City, CA). Generated sequences were compared with the GenBank data bank using the Advanced Blast and IgBlast search programs from the National Center for Biotechnology Information, National Institutes of Health.

Renal histopathology

One kidney from each mouse was obtained at sacrifice on wk 16, and embedded in paraffin. Histological sections were obtained by microtome, and stained with H&E and periodic-acid Schiff (PAS) using standard meth-
ods. Kidney histology was evaluated blindly by an experienced pathologist.
(M. Edelman), without knowledge of the treatment assignment. Histopathological changes in renal tissue were quantitated using the scoring method described by Corna et al. (16), with slight changes. Presence of endocapillary disease was scored from 0 to 3 (0; absent; 1; mild; 2; moderate; 3; severe). The presence of crescents, tubular changes (atrophy, casts, dilatation), and interstitial changes (inflammation, fibrosis) was each graded on a scale of 0–3, with 0, absent; 1, present in <25% of the section; 2, present in 25–50% of the section; and 3, present in >50% of the section. The maximum score for each mouse was 12 (maximum 3 points each for endocapillary glomerular changes, crescent formation, tubular changes, and interstitial damage).

Results

Immunization with a dsDNA surrogate elicits anti-peptide Abs and accelerates autoimmune production in B/W mice

To investigate a possible role of peptide Ag in accelerating autoantibody production in genetically predisposed mice, we immunized preautoimmune B/W mice with a peptide dsDNA surrogate (DWEYSVWLSN), and compared the induced responses with mice immunized with control peptides (SVIWSWMWL and TIALKWLRLWA), and with age- and sex-matched unmanipulated B/W mice. As there were no significant differences between the mice immunized with the different control peptides, these two groups are considered together as MAP-control for the purpose of the analysis. The IgM anti-peptide response began to appear after the second immunization, at wk +3. All MAP-DWEYSVWLSN-immunized mice developed an IgG anti-DWEYSVWLSN response, which was significantly higher than control-immunized mice at wk +5 (p < 0.025), wk +10 (p < 0.0001), and wk +16 (p < 0.0001) (Fig. 1A). Mean IgG anti-cardiolipin titers in MAP-DWEYSVWLSN-immunized mice were significantly higher than control-immunized mice at wk +10 (p = 0.0009) and wk +16 (p = 0.02) (Fig. 1B). Mean IgG anti-dsDNA Ab titers in MAP-DWEYSVWLSN-immunized mice were significantly higher than control-immunized mice at wk +10 (p = 0.009) and wk +16 (p = 0.02) (Fig. 1C). Mean anti-laminin titers in MAP-DWEYSVWLSN-immunized mice were significantly higher than control-immunized mice at wk +5 (p = 0.04), wk +10 (p = 0.005), and wk +16 (p = 0.03) (Fig. 1D). Although anti-laminin titers in MAP-DWEYSVWLSN-immunized mice were higher at baseline, there was no difference between the groups at wk +3, after which the anti-laminin titers began to diverge. IgG anti-histone titers did not differ significantly between MAP-DWEYSVWLSN-immunized and control mice; IgG anti-Sm/RNP Ab titers were higher in the MAP-DWEYSVWLSN-immunized mice only at a single time point (wk +13) (data not shown).

To assess whether the autoimmune titers induced in immunized B/W mice differ from the background titers in mice with spontaneous lupus, we evaluated MAP-DWEYSVWLSN-immunized (n = 14), MAP-control-immunized (n = 9), and unmanipulated B/W mice (n = 8) at 26 wk of age (Fig. 1E). IgG anti-peptide titers in MAP-DWEYSVWLSN-immunized B/W mice were significantly higher than in MAP-control-immunized and unmanipulated B/W mice (p < 0.00001). IgG anti-dsDNA Ab levels in MAP-DWEYSVWLSN-immunized B/W mice (by OD) were significantly higher than MAP-control-immunized B/W mice (p = 0.04). IgG anti-dsDNA Ab levels in MAP-DWEYSVWLSN-immunized B/W mice were also higher than in unmanipulated B/W mice, but the difference did not reach statistical significance (p = 0.114). IgG anti-cardiolipin titers in MAP-DWEYSVWLSN-immunized B/W mice were significantly higher than in MAP-control-immunized (p = 0.0009) and unmanipulated B/W mice (p = 0.02). Finally, IgG anti-laminin titers in MAP-DWEYSVWLSN-immunized B/W mice were significantly higher than in MAP-control-immunized (p = 0.008) and unmanipulated B/W mice (p = 0.016).

The difference in IgG anti-dsDNA Ab levels between the MAP-DWEYSVWLSN-immunized, MAP-control-immunized, and unmanipulated B/W mice groups was further quantitated in titration experiments (Fig. 2A). MAP-DWEYSVWLSN-immunized mice had an IgG anti-dsDNA titer of 12,357 ± 2,558 (mean ± SEM) at 26 wk of age, compared with titers of 2,156 ± 1,325 in MAP-control-immunized mice and 4,450 ± 1,888 in unmanipulated B/W mice (Fig. 2B). The difference in endpoint anti-dsDNA titers between MAP-control-immunized and unmanipulated B/W mice groups was not significant (p = 0.34; Fig. 2B).

To determine whether peptide immunization leads to skewing of autoantibody isotypes, we evaluated the isotype distribution of the anti-peptide and anti-dsDNA Ab responses in MAP-DWEYSVWLSN-immunized, MAP-control-immunized, and unmanipulated B/W mice. As shown in Fig. 3A, DWEYSVWLSN immunization resulted in an IgG1, IgG2a, and IgG2b anti-peptide response. The increase in anti-DWEYSVWLSN Abs in MAP-DWEYSVWLSN vs control-immunized B/W mice was significant for the IgG1 (p = 0.0007), IgG2a (p = 0.015), and IgG2b isotypes (p = 0.004; Fig. 3A), while there was no increase in the IgG3 or IgM anti-peptide responses. The titer of IgG1 (p = 0.002), IgG2a (p = 0.024), and IgG2b (p = 0.005) anti-peptide Abs in MAP-DWEYSVWLSN-immunized B/W mice was significantly higher than in unmanipulated B/W mice of the same age, but did not differ significantly for the IgG3 or IgM isotypes (Fig. 3A). The increase in anti-dsDNA Abs in MAP-DWEYSVWLSN vs control-immunized B/W mice was significant for the IgG2a (p = 0.008), IgG2b (p = 0.044), and IgG3 (p = 0.046) isotypes, but not for IgG1 or IgM (Fig. 3B). The titer of IgG2a (p = 0.048) and IgG3 (p = 0.021) anti-dsDNA Abs in MAP-DWEYSVWLSN-immunized B/W mice was significantly higher than in unmanipulated B/W mice of the same age, but did not reach statistical significance for the IgG1, IgG2b, and IgM isotypes (Fig. 3B). In MAP-DWEYSVWLSN-immunized mice, MAP-DWEYSVWLSN inhibited 50% of the IgG3 anti-dsDNA response, followed by IgM (35%), IgG2b (26%), and IgG2a (16%). Interestingly, the IgG1 anti-dsDNA response that was induced by MAP-DWEYSVWLSN immunization was not inhibited by peptide (Fig. 4A).

To confirm that the increase in anti-peptide and autoantibody titers in peptide-immunized B/W mice was a specific response to DWEYSVWLSN immunization rather than part of a polyclonal response, we measured the total serum Ig levels of IgM, IgG, and the different IgG subclasses. Serum levels of total IgM and IgG, as well as each of the IgG subclasses IgG1, IgG2a, IgG2b, and IgG3, were not different between the MAP-DWEYSVWLSN and control-immunized B/W mice groups at 29 wk of age (data not shown).

MAP-DWEYSVWLSN immunization promotes renal disease

Kidney histopathology was evaluated in 29-wk-old, peptide-immunized B/W mice to determine whether an immune response to peptide can increase the severity of target organ damage in murine lupus. Kidney histology in MAP-DWEYSVWLSN-immunized B/W mice was compared with age-matched, unmanipulated, and control-immunized B/W mice (Fig. 5). Tubular and interstitial damage (p = 0.02) as well as the total kidney damage (which includes the glomerular, crescents, tubular, and interstitial components of the score) (p = 0.02) were significantly greater in DWEYSVWLSN-immunized B/W mice than in unmanipulated B/W mice (Wilcoxon rank sum procedure, Kruskal-Wallis test). Thus, MAP-DWEYSVWLSN-immunized B/W mice had significantly more advanced renal disease than unmanipulated mice.
More severe kidney disease was also apparent in MAP-DWEYSVWLSN-immunized B/W mice in comparison with control-immunized mice, although the differences were less significant (p > 0.05 for tubular and interstitial damage, and p > 0.08 for total kidney damage (Wilcoxon rank sum procedure, Kruskal-Wallis test)). Among the different mouse groups, significant glomerular disease was seen only in MAP-DWEYSVWLSN-immunized B/W mice. Seven of 14 mice in the MAP-DWEYSVWLSN-immunized group demonstrated moderate or severe glomerular disease (a combined glomerular and crescents score of ≥3, range 3–5) as compared with 0 of 7 control-immunized B/W mice (p < 0.05, Fisher’s exact test), and 0 of 5 unmanipulated B/W mice (p = 0.11, Fisher’s exact test). The differences in the total kidney damage score and each of its components between control-immunized and unmanipulated B/W mice were not significant.

**FIGURE 1.** Mean (±SEM) IgG anti-peptide and autoantibody responses in peptide-immunized and unmanipulated B/W mice. Thirteen-week-old B/W mice were immunized with MAP-DWEYSVWLSN (n = 14) or MAP-control peptide (n = 9) in CFA, and boosted with 100 μg of MAP-DWEYSVWLSN or MAP control in IFA after 3, 6, and 9 wk. A, IgG anti-peptide. B, IgG anti-cardiolipin. C, IgG anti-dsDNA. D, IgG anti-laminin. E, Comparison of IgG anti-peptide and autoantibody titers in 26-wk-old MAP-DWEYSVWLSN-immunized (n = 14), MAP-control-immunized (n = 9), and age- and sex-matched unmanipulated (n = 8) B/W mice. An * appears over time points in which the difference between MAP-DWEYSVWLSN- and MAP-control-immunized mice is statistically significant; the # appears over time points in which the difference between MAP-DWEYSVWLSN-immunized and unmanipulated B/W mice is statistically significant (see text for details).
Cross-reactive anti-DNA/anti-peptide Abs are elicited by peptide immunization

Twenty-one mAbs reactive with peptide and/or autoantigen were derived from peptide-immunized B/W mice, all utilizing the \( \kappa \) L chain (IgM-14, IgG-7 (IgG1-1, IgG2a-2, IgG2b-3, IgG3-1) (Table I). As selection of positive hybridomas for further analysis was performed using labeled anti-IgM and anti-IgG in combination as the detection reagent, selection may have been biased toward selecting IgM hybridomas due to higher Ab avidity. Two major groups can be distinguished between these mAb, based upon specificity for peptide: 1) 7 mAbs that do not bind peptide (17-15, 18-2, 27-7, 28-22, 3-4, 30-4, 30-2), and 2) 14 mAbs that bind peptide as well as one or more autoantigens. Abs reactive against peptide alone could not be isolated. Fourteen of the twenty-one mAbs isolated were polyreactive, as defined by their binding to peptide, dsDNA, and at least one other autoantigen (Table I). To address the possibility that polyreactivity may be due to nonspecific binding, we studied whether these polyreactive Abs would bind other, nonlupus-related Ags. As shown in Table II, Ab 19-2 reacted strongly with all of the irrelevant Ags. The other polyreactive mAbs displayed for the most part low-affinity binding, and reacted selectively with the panel of irrelevant Ags. DNA binding of polyreactive Abs could be significantly inhibited by peptide (Fig. 4B), suggesting that cross-reactivity was a property of the Ag-binding site, rather than mediated via a nonspecific charge interaction. Despite a large number of surviving hybrids from the fusion of two spleens of age-matched unmanipulated B/W mice, peptide- and/or dsDNA-specific clones could not be isolated from these mice at 20 wk of age.

To confirm that the antinuclear Ag positivity of the isolated mAbs was not due to nucleosomes present in the hybridoma supernatants, we purified selected IgM (17-15, 25-32, 21-28, 11-6,
The pathogenic anti-DNA response in systemic lupus is T cell dependent. Anti-dsDNA Abs that are most closely clinically associated with SLE, that can be eluted from sites of active organ damage such as the kidney, and that display pathogenic potential in different in vitro systems are generally IgG, have high affinity for DNA, and display somatic mutations throughout the H and L chain V regions (2, 3). Furthermore, inhibiting T cell function in the B/W murine lupus model is therapeutic, and greatly attenuates disease manifestations (17). Although immunization with mammalian DNA alone (not in association with a DNA-binding protein) is not sufficient to induce an anti-DNA response in murine models (7, 18), immunization with complexes of DNA and DNA-binding proteins can elicit an anti-dsDNA response with similarities to the anti-DNA Abs present in lupus (9, 11, 19). Presumably in these models, T cells specific for the protein component of the complex facilitate anti-DNA Ab production by DNA-specific B cells. We have previously reported the isolation from a phage display library of DWEYSVWLSN, a peptide surrogate for dsDNA, that was sufficient through molecular mimicry (without additional complexing with DNA) to induce an anti-DNA Ab response in BALB/c mice (12). However, although renal Ig deposition was present in these mice, histological examination by light microscopy did not reveal overt glomerulonephritis.

We have examined in this study the effect of immunization with the peptide DNA surrogate on the immune response and disease course of mice genetically predisposed to autoimmunity, and we have analyzed the molecular genetics of their anti-dsDNA response. B/W mice spontaneously begin to produce anti-dsDNA Abs at 26–30 wk of age and develop clinical nephritis at 36–40 wk (20). Control-immunized B/W mice appeared to be following this schedule. In contrast, MAP-DWEYSVWLSN-immunized mice developed significantly higher IgG anti-DNA, anticardiolipin, and anti-laminin Abs starting from ~10 wk after the initial exposure to peptide, and continuing until the mice were sacrificed at 29 wk of age.

Several important differences in the immune and autoimmune responses to peptide between nonautoimmune BALB/c and autoimmune B/W mice are worth emphasizing. B/W mice had a somewhat delayed anti-peptide response to DWEYSVWLSN. We previously found that the response to DWEYSVWLSN is genetically determined, with several mouse strains not mounting any anti-peptide response to immunization (12). The short delay in the anti-

![FIGURE 4](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org)
peptide response in B/W mice may be a result of a genetic variability in the response to this peptide. Alternatively, it is possible that the initial peptide immunizations elicited a cross-reactive autoantibody response, which was effectively down-regulated when the B/W mice were young. Peptide-immunized B/W mice, in contrast to our earlier findings in BALB/c mice, did not display a consistent increase in serum IgG anti-Sm/RNP Abs, presumably reflecting the general absence of Abs with these specificities in the B/W lupus strain. In peptide-immunized BALB/c mice, both the anti-peptide as well as the anti-DNA Abs were predominantly of the IgG1 isotype. Moreover, in peptide-immunized BALB/c mice, IgG1 was prominent in renal immune deposits (12). In serum of immunized B/W mice, however, anti-peptide and anti-DNA Abs of the IgG2a and IgG2b isotypes were present. This is consistent with spontaneous lupus in B/W mice, in which anti-DNA Abs of the IgG2a and IgG2b isotypes are most frequent, and the predominant isotype in glomerular deposits is IgG2a (21). The induced isotypes of the anti-dsDNA Ab response in peptide-immunized B/W mice, as opposed to peptide-immunized BALB/c mice, may have contributed to the more severe renal disease induced in B/W mice in response to peptide immunization (22).

Comparison of the isotypes in the induced anti-peptide and anti-dsDNA Ab responses in MAP-DWEYSVWLSN-immunized mice with the spontaneous response in unmanipulated B/W mice reveals some notable differences. The induced anti-MAP-DWEYSVWLSN response in immunized mice, as compared with unmanipulated mice, is primarily IgG1, IgG2a, and IgG2b; this suggests that the anti-peptide response results from activation of follicular B cells, which undergo maturation and class switching in germinal centers. In the isotype analysis of the anti-dsDNA response, however, IgG2a as well as...
IgG3 anti-dsDNA Abs were significantly higher than in mice with spontaneous disease. Perhaps the most important difference between MAP-DWEYSVWLSN-immunized BALB/c and B/W mice was found in analysis of target organ damage in the kidney. Although glomerular immune deposits occurred in peptide-immunized BALB/c mice, none of the mice displayed any histologic evidence of renal damage at light microscopy resolution (12). In contrast, DWEYSVWLSN immunization precipitated severe glomerular and to a lesser extent tubulointerstitial disease that was not present in B/W mice of the same age immunized with control peptides. Similarly, global kidney damage (as measured by a composite of the damage scores in the glomerular, crescents, tubules, and interstitium categories) was significantly greater in MAP-DWEYSVWLSN-immunized than in unmanipulated B/W mice.

Table II. Antigenic specificity of polyreactive Abs for lupus-irrelevant Ags

<table>
<thead>
<tr>
<th>Isotype</th>
<th>dsDNA</th>
<th>ssDNA</th>
<th>Peptide</th>
<th>Cardiolipin</th>
<th>Histone</th>
<th>Sm/RNP</th>
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<td>B/W3,14-1</td>
<td>IgM</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>0.92</td>
<td>1.67</td>
</tr>
<tr>
<td>B/W1,21-28</td>
<td>IgM</td>
<td>0.44</td>
<td>0.87</td>
<td>1.33</td>
<td>0.77</td>
<td>1.14</td>
</tr>
<tr>
<td>B/W2,24-13</td>
<td>IgM</td>
<td>1.45</td>
<td>2.39</td>
<td>2.48</td>
<td>2.35</td>
<td>0.49</td>
</tr>
<tr>
<td>B/W3,11-6</td>
<td>IgM</td>
<td>3.5</td>
<td>3.5</td>
<td>1.08</td>
<td>1.06</td>
<td>0.15</td>
</tr>
<tr>
<td>B/W2,24-4</td>
<td>IgM</td>
<td>0.67</td>
<td>1.52</td>
<td>0.68</td>
<td>1.56</td>
<td>0.12</td>
</tr>
<tr>
<td>B/W2,19-19</td>
<td>IgG1</td>
<td>0.31</td>
<td>0.76</td>
<td>1.66</td>
<td>1.58</td>
<td>0.75</td>
</tr>
<tr>
<td>B/W2,3-4</td>
<td>IgG2a</td>
<td>3.5</td>
<td>3.5</td>
<td>0.12</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>B/W2,30-4</td>
<td>IgG2a</td>
<td>0.1</td>
<td>0.16</td>
<td>0.22</td>
<td>1.54</td>
<td>0.14</td>
</tr>
<tr>
<td>B/W3,29-2</td>
<td>IgG2b</td>
<td>0.36</td>
<td>1.0</td>
<td>2.04</td>
<td>1.79</td>
<td>1.05</td>
</tr>
<tr>
<td>B/W3,20-3</td>
<td>IgG2b</td>
<td>3.5</td>
<td>2.7</td>
<td>1.05</td>
<td>1.52</td>
<td>3.5</td>
</tr>
<tr>
<td>B/W3,9-18</td>
<td>IgG2b</td>
<td>0.24</td>
<td>0.42</td>
<td>1.58</td>
<td>0.93</td>
<td>0.46</td>
</tr>
<tr>
<td>B/W2,30-2</td>
<td>IgG2b</td>
<td>0.41</td>
<td>0.63</td>
<td>0.76</td>
<td>0.26</td>
<td>0.31</td>
</tr>
</tbody>
</table>

It is important to note that several of the peptide-immunized mice displayed significant tubulointerstitial disease, including infiltration by mononuclear cells. Although the histopathological lesion in lupus with the greater physiological consequence to renal function is glomerular disease, tubulointerstitial inflammation is quite common in both human (23) and murine (24, 25) lupus nephritis. In a study by Hurd and Ziff (24), 100% of B/W mice at 7 mo of age displayed significant mononuclear cell interstitial infiltration. Therefore, we believe that the interstitial renal disease as well as the glomerulopathy observed in peptide-immunized B/W mice might be related to this immune response.

Table III. VH and Vκ usage in mAbs derived from MAP-DWEYSVWLSN-immunized B/W mice

<table>
<thead>
<tr>
<th>dsDNA/Peptide</th>
<th>VH</th>
<th>D1H</th>
<th>JH</th>
<th>Vκ</th>
<th>Ik</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/W1,19-2</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.1</td>
<td>2</td>
<td>VOκ-1</td>
</tr>
<tr>
<td>B/W1,17-15</td>
<td>+/+</td>
<td>J558</td>
<td>DQ52</td>
<td>3</td>
<td>VK1-b</td>
</tr>
<tr>
<td>B/W2,18-2</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.2c</td>
<td>3</td>
<td>VK1-a</td>
</tr>
<tr>
<td>B/W2,27-7</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.2c</td>
<td>3</td>
<td>VK1-b</td>
</tr>
<tr>
<td>B/W2,19-23</td>
<td>+/+</td>
<td>J558</td>
<td>DQ52</td>
<td>2</td>
<td>VK1</td>
</tr>
<tr>
<td>B/W2,25-32</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.2c</td>
<td>3</td>
<td>VK1-c</td>
</tr>
<tr>
<td>B/W3,12-5</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.2c</td>
<td>3</td>
<td>VK1-a</td>
</tr>
<tr>
<td>B/W2,28-22</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.1c</td>
<td>2</td>
<td>VK1-a</td>
</tr>
<tr>
<td>B/W3,14-1</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.1b</td>
<td>3</td>
<td>VK1-c</td>
</tr>
<tr>
<td>B/W2,19-19</td>
<td>+/+</td>
<td>J558</td>
<td>DSP2.6</td>
<td>4</td>
<td>VK4</td>
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<tr>
<td>B/W2,3-4</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.2</td>
<td>3</td>
<td>VK1-a</td>
</tr>
<tr>
<td>B/W2,30-4</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.2c</td>
<td>3</td>
<td>VK1-a</td>
</tr>
<tr>
<td>B/W3,29-2</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.3</td>
<td>3</td>
<td>VK1-c</td>
</tr>
<tr>
<td>B/W3,30-3</td>
<td>+/+</td>
<td>J558</td>
<td>DSP2.9</td>
<td>4</td>
<td>VK4</td>
</tr>
<tr>
<td>B/W3,9-18</td>
<td>+/+</td>
<td>J558</td>
<td>DSP2.6</td>
<td>3</td>
<td>VK32</td>
</tr>
<tr>
<td>B/W2,30-2</td>
<td>+/+</td>
<td>J558</td>
<td>DFL1.16.2</td>
<td>4</td>
<td>VK4</td>
</tr>
</tbody>
</table>

* Normalized supernatants at 10 μg/ml were incubated on Ag-coated plates for 2 h, followed by the appropriate alkaline phosphatase-linked goat anti-mouse Ig and substrate. Numbers reflect OD readings at 405 nm. mAbs are defined as positive (in bold) for a given specificity if the OD value was greater than the average of at least two isotype-matched control Abs plus 2 SD.
mice do not represent a novel form of kidney disease, but rather significant acceleration of the type of nephritis due to appear later on in the course of spontaneous murine lupus.

Although anti-histone Abs were not induced in MAP-DWEYSVWLNS-immunized mice, both IgG anti-DNA and IgG anti-laminin Ab titers were significantly higher than in controls. The latter Ab specificities have been closely associated with renal pathogenicity in lupus (2-4, 26), and probably contributed to the accelerated renal disease seen in immunized mice. Although control-immunized and unmanipulated B/W mice also had measurable autoantibodies in their serum, the titers were lower than in MAP-DWEYSVWLNS-immunized mice.

IgG is the Ab class most closely associated with kidney disease in lupus (2). It is interesting to note that IgG autoantibodies from peptide-immunized B/W mice have been more easily isolated than from BALB/c mice, suggesting that induced pathogenic Abs are more frequent and/or regulated differently in peptide-immunized B/W mice. Differences in antigenic specificity, isotype, or complement-fixating capability of the induced Abs, the cytokine milieu, or T cells may also be possible contributors to the observed pathologic differences between BALB/c and B/W peptide-immunized mice. Alternatively, genetic differences in Ag display at the level of the kidney are responsible for the differing degree of renal damage. These possibilities will be addressed directly in future studies.

Molecular genetic analysis of the peptide and autoantibody responses in B/W mice reveals some novel features. With respect to H chain gene usage, all mAbs showed close homology to spontaneously arising anti-DNA Abs from B/W mice. The H chains of two mAbs (14-1 and 30-4) showed, in addition, close homology to the H chains of F1-3 and F5-40, anti-DNA Abs induced in preautoimmune B/W mice by bacterial DNA immunization (18), respectively. Furthermore, the H chains of eight mAbs (25-32, 11-6, 12-5, 29-2, 24-4, 7-13, 3-4, and 9-18) displayed in addition significant similarities to H chains used in anti-peptide and autoantibodies arising in nonautoimmune BALB/c mice immunized with MAP-DWEYSVWLNS (13). Two-thirds (14 of 21) of the mAbs used a J558 family member to encode for the H chain V region of peptide- and/or DNA-binding Abs in peptide-immunized B/W mice as compared with only 33% (8 of 24) in peptide-immunized BALB/c mice (13), apparently in conformance with the J558 bias in spontaneously arising anti-DNA Abs in B/W mice (27). Q52 (4 of 21 in B/W, 3 of 24 BALB/c) and Vg1-0-derived H chains (1 of 21 B/W, 3 of 24 BALB/c) showed less of a difference in usage frequency between strains. Several H chain gene families represented in the BALB/c response to peptide (S107, 7183, 36-60, 606) were not isolated from mAbs in B/W mice. Although unusual D segment utilization has been reported in murine lupus anti-DNA Abs (28, 29), D-D fusions or inverted Ds were not identified in peptide-immunized B/W mice.

The L chain of all but four mAbs (14-1, 21-28, 24-4, and 3-4) were closely homologous to L chains used in the spontaneous anti-DNA response in B/W mice. The L chain of Ab 14-1 used a Vk1-c-derived gene segment. The L chains of Abs 21-28 and 24-4 were identical, and were closely related to the L chain of bd05, an induced anti-DNA Ab derived from a BALB/c mouse immunized with the Fus1 peptide (19). The 3-4 L chain was closely related to the L chain of the 84-39 anti-DNA Ab arising in a transgenic BALB/c mouse (30). Light chain subfamily usage did not appear to significantly differ between B/W- and BALB/c-immunized mice: a Vk1-a-encoded L chain was used in two mAbs in B/W mice and three mAbs in BALB/c mice, a Vk1-c-encoded L chain was used in three mAbs in B/W mice and two mAbs in BALB/c mice, and a Vk1-b L chain in a single B/W-derived mAb.

Nine mAbs (of which eight bound DNA) displayed V\(_{\text{H}}\)-V\(_{\text{L}}\) combinations common in the spontaneous anti-DNA response in B/W mice (27): six mAbs displayed a J558-Vk1 combination, and three mAbs had a J558-Vk8 combination. Interestingly, of the six J558-Vk1 Abs that bound DNA, only 14-1 and 29-2 also bound peptide. Since J558-Vk1 and J558-Vk8 combinations appeared only in 3 of 24 Abs in peptide-immunized BALB/c mice.

Since the H chains from the MAP-DWEYSVWLNS-immunized mAbs are encoded by the same genes that are used in spontaneously arising anti-DNA Abs in B/W mice, it is of particular interest to analyze those mAbs from peptide-immunized mice that do not bind DNA (Table I). Comparison with mAbs 165.3 and 165.6 (27), previously described anti-DNA Abs from B/W mice, is particularly pertinent. The V and J regions of 18-2 and 165.3 H chains are identical; however, the D region of 165.3 contains two arginines, an amino acid that is important in conferring anti-DNA specificity. The 30-4 H chain has a single FR1 substitution as compared with 165.6, and is otherwise identical through FR3. The D region of 165.6 is 10 aa long and has several charged residues, while the D region of 30-4 is much shorter, and not charged. An alternative explanation for the lack of binding to DNA by these mAbs may lie in the L chain. Although the L chains of 18-2 and 30-4 are closely homologous or identical to L chains of DNA-binding Abs, it is possible that small differences in the L chain or in the specific H and L chain paring contribute to the loss of specificity for DNA. Nevertheless, to conclusively demonstrate which amino acids are important in DNA binding, and whether peptide or DNA binding is encoded in the germline, further studies by mutation analysis are necessary.

Results in B/W mice reported in this study are consistent with our findings in analysis of the induced response in peptide-immunized BALB/c mice, namely, that autoantibodies induced in response to peptide immunization show close structural homology to anti-DNA Abs arising in spontaneous murine lupus. Interestingly, this has also been the conclusion of analyses of anti-DNA Abs isolated in two induced disease models that are quite different from our peptide-induced model, specifically immunization with Fus1 (a DNA-binding peptide from Trypanosoma cruzi) in complex with DNA (9) and bacterial DNA (18). Furthermore, Abs induced in the anti-peptide response are homologous to induced anti-DNA Abs in the latter two models. Taken together, these results seem to indicate a common molecular genetic response pathway to a variety of stimuli that share the ability to break tolerance to native, mammalian DNA.

Most of the mAbs induced by immunization of B/W mice with the peptide DNA surrogate were cross-reactive, binding both peptide and dsDNA (Table I). We isolated five anti-DNA mAb that do not also bind peptide: 17-15, 27-7, 28-22, 3-4, and 30-2 (Table I). Of these mAb, the H chains of 17-15, 27-7, 28-22, and 3-4 are closely related, and probably derive from the same germline gene as 111.185, an IgM anti-DNA Ab derived from a B/W mouse (27). Although it is not known whether the 111.185 Ab binds peptide, the fact that the H chains of 17-15, 27-7, 28-22, and 3-4 are related, yet the mAbs do not bind peptide suggests that it is possible that some anti-DNA Abs in peptide-immunized B/W mice arose spontaneously, not in response to peptide. Consistent with this suggestion is the finding that while peptide partially inhibited the binding of sera from peptide-immunized B/W mice to dsDNA, the degree of inhibition was less than in peptide-immunized BALB/c mice (12).

How are cross-reactive anti-peptide/anti-DNA Abs being generated in this model? Although we could not isolate germline-encoded, cross-reactive Abs, cross-reactivity may be germline encoded. Alternatively, mutation of spontaneously arising anti-DNA
Abs to acquire specificity for peptide, or somatic mutation in the anti-peptide immune response can account for cross-reactive anti-peptide/anti-DNA Abs in peptide-immunized B/W mice. We hope to be able to find direct evidence for either or both of these hypotheses by selective analysis of the peptide-binding spleenocyte population in peptide-immunized mice.

There are several possible ways to explain the derivation of Abs that are cross-reactive for both histone and dsDNA from peptide-immunized B/W mice, despite the disparity in charge between these two autoantigens. The immunizing peptide DWEYSVWLSN is a peptide mimic of DNA for R4A, an Ab that is specific for DNA as well as histone. In studies from other investigators as well as in our own work (13), it has been demonstrated that immunization with a peptide mimic can generate Abs with binding sites that are similar to the original Ab used to select the peptide from the phage library. Similarly, immunization with the R4A peptide mimotope elicits cross-reactive anti-dsDNA/anti-histone Abs. Furthermore, Monestier et al. (31) reported that certain anti-histone mAbs isolated from lupus-prone mice can bind to DNA in the absence of histone.

In summary, immunization of mice genetically predisposed to autoimmunity with a peptide surrogate of dsDNA induced the early onset of high titer anti-DNA, anti-laminin, and anti-cardiolipin Abs, and precipitated severe lupus-like glomerular and tubulointerstitial disease. Peptide immunization in B/W mice appeared to have resulted in a markedly increased frequency of B cells secreting pathogenic autoantibodies, resulting in accelerated disease. Interestingly, in only 1 of 14 sera from MAP-DWEYSVWLSN-immunized B/W mice there was any inhibition of the anti-cardiolipin and anti-laminin responses by peptide, supporting the suggestion that peptide immunization induces autoimmune manifestations in B/W mice by promoting the underlying disease. Although there were some differences in autoantibody isotypes, the induced anti-dsDNA Abs displayed close structural similarities to anti-DNA Abs arising in spontaneous, as well as induced murine lupus.

Based on the demonstrated similarity between the spontaneous, and several types of induced anti-DNA Abs, it may be possible to postulate that may not be a single, triggering Ag for the disease clinically classified as SLE. Furthermore, although direct evidence for this hypothesis is not available at this time, the similarities between the induced autoimmune response to peptide and spontaneous autoimmunity suggest that peptide Ags may induce an autoimmune response in individuals with certain genetic predispositions. Finally, peptides that can up-regulate the autoantibody response in lupus have been used successfully in blocking kidney deposition of Ig, delaying the onset of renal disease, and in prolonging survival in murine lupus. Possible mechanisms to explain this therapeutic benefit include induction of Ag-specific unresponsiveness, activation of regulatory T cells, or interference with binding to a cross-reactive antigenic target (32). Similarly, peptide DNA mimotopes that induce lupus-like autoantibodies by immunization may eventually turn out to have therapeutic potential in SLE.

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References