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Fas and Fas Ligand Mutations Inhibit Autoantibody Production in Pristane-Induced Lupus

Minoru Satoh,* Jory P. Weintraub,† Hideo Yoshida,* Victoria M. Shaheen,‡ Hanno B. Richards,* Melody Shaw,† and Westley H. Reeves‡∗

Mutations of Fas (lpr) or Fas ligand (gld) cause a limited lupus-like syndrome in B6 mice by interfering with the deletion of autoreactive B and/or T cells. A more generalized lupus syndrome reminiscent of that of MRL mice can be induced in nonautoimmune strains by pristane, which causes a nonspecific inflammatory response in the peritoneal cavity. We hypothesized that, as in MRL mice, the lpr and gld mutations might accelerate lupus in pristane-treated mice. Pristane-treated B6 mice developed anti-nRNP/Sm, Su, and ribosomal P Abs, but little anti-ssDNA or chromatin. In contrast, B6/lpr and B6/gld mice spontaneously developed anti-ssDNA/chromatin Abs, but not anti-nRNP/Sm/Su/ribosomal P. Unexpectedly, B6/lpr and B6/gld mice were highly resistant to the induction by pristane of IgM anti-ssDNA (2 wk) and IgG anti-nRNP/Sm/Su/ribosomal P autoantibodies (6 mo), suggesting that intact Fas signaling is necessary. Interestingly, pristane did not enhance IgG chromatin Ab production in B6/lpr or B6/gld mice, suggesting that it did not influence the production of autoantibodies that develop spontaneously in the setting of Fas deficiency. Pristane treatment also decreased lymphoproliferation in B6/lpr mice. Increased production of IL-12 was associated consistently with the production of anti-nRNP/Sm/Su/ribosomal P as well as anti-DNA/chromatin. In contrast, production of anti-DNA/chromatin Abs was associated with IL-6 overproduction in pristane-treated mice, but not in lpr mice. The data strongly support the idea that different subsets of autoantibodies are regulated differentially by cytokine stimulation and/or Fas signaling. The Journal of Immunology, 2000, 165: 1036–1043.

S
ystemic lupus erythematosus (SLE) is a complex genetic disorder, the expression of which is influenced by sun exposure and other poorly characterized environmental triggers (1, 2). It is believed that the environmental triggers act only in concert with an autoimmune-prone, genetic background. Thus, exposure of nonautoimmune-prone individuals to UV radiation does not precipitate lupus, whereas it does in susceptible individuals. Pristane-induced lupus in nonautoimmune mice is a useful model for examining the role of environmental triggers in autoimmunity (3, 4). In the present study, we investigated the interaction between a nonspecific inflammatory trigger of lupus (pristane) and genetic defects that promote lupus by interfering with activation-induced cell death.

Mutations of the Fas (lpr) or Fas ligand (gld) genes impair activation-induced cell death (5–7), resulting in abnormal peripheral deletion of autoreactive T and B cells and the development of a lupus-like syndrome characterized by anti-ssDNA and anti-chromatin autoantibodies and lymphoproliferation (8). On autoimmune backgrounds such as MRL (5) or BXSB (9), the lpr mutation greatly accelerates lupus. In view of the abnormal peripheral deletion of autoreactive T and B cells resulting from these mutations and the autoantibodies produced spontaneously by B6/lpr and B6/gld mice, lpr or gld would be expected to exacerbate autoantibody production in pristane-treated B6 mice. The present studies show that, contrary to expectations, defective Fas-Fas ligand interaction antagonizes the autoimmune syndrome induced by pristane.

Materials and Methods

Treatment of mice

Four-week-old female B6, B6/lpr/lpr, and B6/gld/gld mice were purchased from The Jackson Laboratory (Bar Harbor, ME), and housed in a virus-free conventional animal facility with barrier cages. The lpr and gld double mutant mouse, B6/lpr-gld/gld, was described previously (8). At 3 mo of age, 7–16 mice per group received 0.5 ml i.p. of pristane (Sigma, St. Louis, MO) or sterile PBS (3). Sera were collected from the tail vein before injection, at 2 and 4 wk, and monthly thereafter. Sera from untreated B6/lpr, B6/gld, and B6/lpr-gld double mutant mice also were studied.

Ig levels

Total levels of IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM were measured by sandwich ELISA, as described (10).

Radiolabeling and immunoprecipitation

Autoantibodies to cellular proteins in murine and human sera were analyzed by immunoprecipitation of 35S-radiolabeled K562 cell extract using 3 μl murine serum, as described. Specificity of autoantibodies was confirmed using human reference sera for anti-nRNP/Sm, anti-Su, and anti-ribosomal P.

ELISAs for autoantibodies to nRNP/Sm, ssDNA, and chromatin

A standard ELISA for anti-nRNP/Sm was used (11). Sera were tested at 1/500 dilution, and OD450 was converted to units using a standard curve based on Y2 anti-Sm mAb, as described (11). The anti-ssDNA Ab ELISA was performed, as described, using heat-denatured calf thymus DNA (Sigma) as Ag (4). IgM anti-ssDNA levels between groups were compared as a group because of the occasional presence of this specificity in sera from

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normal mice. The ratio of increase in IgM anti-ssDNA Abs was calculated as follows: OD405 of sera 2 wk after pristane treatment / OD405 of sera before treatment. IgG anti-ssDNA Ab levels were considered positive when OD405 of the sample was higher than the mean + 3 SD of sera from PBS-treated mice.

Anti-chromatin Abs were measured as described (12), with minor modifications. Wells of microtiter plates (Nunc, Maxisorp, Naperville, IL) were coated with 100 μl of 1 mg of chicken chromatin in borate-buffered saline (200 mM boric acid, 75 mM NaCl, pH 8.4) at 4°C for 16 h. Wells were washed with NET/Nonidet P-40 (0.15 M NaCl, 2 mM EDTA, 50 mM Tris-HCl, pH 7.5, 0.5% Nonidet P-40) and blocked with 0.5% BSA in NET/Nonidet P-40 for 1 h at 22°C. Wells were then incubated with 100 μl of 1/500 mouse sera in same buffer for 1 h at 22°C, washed three times with NET/Nonidet P-40, and incubated with 100 μl of alkaline phosphatase-labeled goat anti-mouse IgG (1/1000) in blocking buffer for 1.5 h at 22°C. After washing, the plates were developed with p-nitrophenyl phosphate (Sigma). OD 405 was converted to units based on a standard curve produced by serial dilutions of pooled sera from MRL/lpr mice: 1/500 dilution = 1,000 U; 1/5000 = 100 U; 1/50,000 = 10 U; 1/500,000 = 1 U. Usually, the standard is clearly positive at a 1/50,000,000 dilution. Because anti-chromatin Abs are produced sporadically in some nonautoimmune strains of mice, the mean + 3 SD of 10 blank wells was used as cutoff for positive and negative.

Tissues and peritoneal lavage

Mice were euthanized with CO2, and the peritoneal cavity was lavaged with 5 ml of high-glucose DMEM + 10% FCS and 1 U/ml heparin using a 5-ml plastic syringe and 18-gauge needle. Samples were kept on ice before centrifuging at 1200 rpm for 10 min. Aliquots of the supernatant were frozen at −80°C. After peritoneal lavage, the liver, spleen, kidney, lymph nodes (cervical, axillary, inguinal, and paraaortic), and thymus/mediastinal lymph nodes were harvested and weighed.

Cytokine ELISA

ELISAs for IL-6, IL-10, IL-12, and TNF-α were performed using rat mAb pairs for various mouse cytokines (PharMingen, San Diego, CA). After incubation with biotinylated cytokine-specific Abs, 100 μl/well of 1/1000 streptavidin-alkaline phosphatase (Southern Biotechnology Associates, Birmingham, AL) was added for 30 min at 22°C, and the reaction was developed.

Statistical analysis

Frequencies of autoantibodies were compared by Fisher’s exact test. The Kruskal-Wallis test and Mann-Whitney tests were used to compare levels of Abs or cytokines (ELISA), and organ weights.

Results

Pristane-induced lupus in nonautoimmune mice resembles the genetically determined lupus occurring spontaneously in MRL mice. Anti-nRNP/Sm, Su, ribosomal P, ssDNA, dsDNA, and chromatin autoantibodies are produced, and the mice develop immune complex-mediated glomerulonephritis (3, 4). Because lupus is exacerbated in MRL and BXSB mice by the lpr or gld mutations (5), we investigated the relationship between these defects and the lupus-like syndrome that develops in the setting of chronic inflammation induced by pristane.

Pristane has little effect on IgG anti-DNA/chromatin Ab production by B6/lpr mice

IgG Abs to ssDNA were present in all PBS-treated B6/lpr and B6/gld mice at 8 mo (5 mo after PBS treatment; Fig. 1A, see below). These Abs were detectable as early as 6 mo of age (3 mo after PBS treatment; not shown). Pristane had little effect on this spontaneous anti-ssDNA autoantibody production, although the levels in pristane-treated B6/lpr mice were somewhat higher than those in PBS-treated group. One of 7 PBS-treated mice vs 7 of 12 pristane-treated mice had IgG anti-ssDNA >1 U, but differences in the levels or frequencies were not significant (Mann-Whitney and Fisher’s exact test, respectively). In contrast, IgG anti-ssDNA Abs were not found in PBS-treated B6 controls. Nor were they increased substantially by pristane treatment (Fig. 1A, Table I).

Anti-chromatin Abs also were not affected significantly by pristane treatment (Fig. 1B). All PBS-treated B6/lpr and B6/gld mice spontaneously produced high levels of IgG anti-chromatin Abs, as expected (5). However, the levels of IgG anti-chromatin Abs were similar in PBS vs pristane-treated B6/lpr and B6/gld mice. In addition, 2 of 8 PBS-treated and 3 of 10 pristane-treated B6 mice had low levels of IgG anti-chromatin Abs (Fig. 1B).

These results suggest that pristane treatment had little effect on the pathogenesis of anti-DNA/chromatin Abs in B6/lpr or B6/gld mice.

Autoantibodies induced in B6 mice by pristane vs lpr or gld

Immunoprecipitation was used to examine other (non-DNA/chromatin) autoantibody responses induced in B6 mice by pristane vs those induced by lpr or gld. Pristane-treated B6 mice produced autoantibodies to the nRNP/Sm (35%), Su (24%), and ribosomal P (12%) Abs (Fig. 2A, Table I) at frequencies comparable with those in MRL/lpr mice or human SLE patients. Two mice also produced autoantibodies to the dsDNA-binding factor NF90/NF45 (13). In striking contrast, none of the B6/lpr, B6/gld, or B6/lpr-gld double mutant mice spontaneously (PBS treated or untreated) produced any of these specificities. Immunoprecipitation of a group of proteins likely to include core histones was common in control B6/lpr (Fig. 2B), B6/gld (Fig. 2C), and B6/lpr-gld double mutant mice (not shown). MRL/lpr mice also produce these autoantibodies (14), but this pattern was not seen in pristane-treated B6 mice, despite the induction of a variety of other lupus autoantibodies (Table I). Abs specific for histones and dsDNA, but not ssDNA, also immunoprecipitated these proteins, arguing that this pattern of interactions is similar to that seen in lupus.
may reflect the presence of a subset of anti-DNA/chromatin Abs (15). These data strongly suggest that pristane enhances/accelerates autoantibody production in a manner different from lpr or gld. This interpretation was supported by studies of the effects of these mutations on the phenotype of pristane-induced lupus.

lpr and gld inhibit induction of anti-nRNP/Sm autoantibodies by pristane in B6 mice

Only one mouse each in the pristane-treated B6/lpr and B6/gld groups produced very low levels of anti-nRNP/Sm Abs by immunoprecipitation, whereas anti-Su, ribosomal P, and NF45/90 were not induced at all (Table II). In contrast, pristane induced anti-nRNP/Sm, Su, or ribosomal P in 3%, 1%, and 2% of B6 mice, respectively (Table I). Production of at least one of these specificities occurred more frequently in B6 mice than in B6/lpr (8/17 vs 1/17, p = 0.0432 by Fisher's exact test). Combining the B6/lpr and B6/gld groups, the increased frequency of autoantibody induction by pristane in B6 was even more obvious (8/17 in B6 vs 2/17 in B6/lpr + B6/gld, p = 0.0232). Taken together with the

Table II. Autoantibodies in pristane-treated B6 mice a

<table>
<thead>
<tr>
<th>Strain</th>
<th>Rx</th>
<th>n</th>
<th>RNP</th>
<th>Su</th>
<th>rib-P</th>
<th>NF90</th>
<th>Any</th>
<th>Histones</th>
<th>ssDNA</th>
<th>Chromatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>PBS</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>+/-</td>
<td>Pristane</td>
<td>17</td>
<td>35</td>
<td>24</td>
<td>12</td>
<td>12</td>
<td>47+</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>lpr/lpr</td>
<td>Pristane</td>
<td>12</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>33</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>gld/gld</td>
<td>Pristane</td>
<td>9</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>33</td>
<td>33</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

a Abs to RNP (nRNP or Sm), Su, ribosomal P, NF90/NF45, and core histones were detected by immunoprecipitation using sera obtained 6 mo after treatment with pristane or PBS (0.5 ml i.p.). For mice that died before that time (mainly gld/gld mice), the latest available monthly serum sample was used. "Any" refers to the detection of one or more of the previous autoantibodies by immunoprecipitation. Abs to ssDNA and chromatin were detected using ELISAs.

* p = 0.04 vs lpr/lpr (Fisher exact test).
lack of enhancement of spontaneous anti-dsDNA or chromatin Ab production by pristane treatment, these data suggest that Fas and Fas ligand mutations make B6 mice refractory to the induction of autoantibodies by pristane.

**Effect of pristane on autoantibody levels**

To see whether *lpr* reduces the levels of autoantibodies induced by pristane as well as their frequency, IgG anti-nRNP/Sm Abs were quantified by ELISA in B6 and B6/*lpr* mice 6 mo after PBS or pristane treatment (Fig. 3). Consistent with the very weak signal by immunoprecipitation, levels of anti-nRNP/Sm Abs were much lower in pristane-treated B6/*lpr* mice than in pristane-treated B6 mice. One B6/*gld* mouse developed anti-nRNP/Sm Abs at very low levels 2 mo after pristane injection, but died before 3 mo (not shown).

**Effects of *lpr* and *gld* on pristane-induced IgM anti-ssDNA Abs**

Pristane rapidly (2 wk after injection) induces the production of IgM anti-ssDNA Abs. Unlike late (1–6 mo after pristane injection) autoantibody production, the early phase probably corresponds to the production of natural autoantibodies (16). To examine the effect of *lpr* and *gld* on the early phase, the increase in IgM anti-ssDNA Abs was determined in sera of individual mice 2 wk after treatment. Ratios of (OD405 2 wk after treatment) / (OD 405 before treatment) for PBS vs pristane-treated B6 and B6/*lpr* mice are shown in Fig. 4. B6 mice treated with pristane averaged a 3-fold increase in IgM anti-ssDNA Abs, whereas little increase was seen in PBS-treated mice (p = 0.019, Mann-Whitney test). In striking contrast, serum IgM anti-ssDNA Abs in pristane-treated B6/*lpr* and B6/*gld* mice did not increase after pristane treatment, suggesting that Fas or Fas ligand mutant mice lack the early pristane-induced autoantibody response.

**Effects of pristane on Ig levels**

IgM and IgG3 levels increase within 2 wk of pristane injection in BALB/cByJ mice in conjunction with the production of IgM anti-ssDNA Abs (4). Similarly, in comparison with PBS-treated controls, total IgM levels increased 2.5-fold (p = 0.0028) and IgG3 levels 2-fold (p = 0.057) within 2 wk after treating B6 mice with pristane (Fig. 5). In contrast, IgM and IgG3 levels were comparable in PBS and pristane-treated B6/*lpr* mice. There were modest, but not statistically significant, increases in IgM and IgG3 in pristane-treated B6/*gld* mice. Taken together, these data suggest that early Ig production is deficient in pristane-treated Fas or Fas ligand mutant mice.

Following the early phase, a polyclonal increase in IgG1, IgG2a, and IgA occurs 1–6 mo after pristane injection in BALB/cByJ mice (10). Five months after treatment, the mean levels of IgG2a (p = 0.03 by Mann-Whitney) and IgG2b (p = 0.03) were ~2-fold higher in pristane-treated B6 mice than in PBS-treated controls (Fig. 5). With the exception of IgG1 in B6/*gld* mice (p = 0.22, not significant), there was little difference in the total IgG1, IgG2a, or IgG2b
levels in B6/lpr and B6/gld mice between the PBS- and pristane-treated groups. Thus, although B6/lpr and B6/gld mice had higher baseline IgG1, IgG2a, and IgG2b levels than B6 mice, pristane had little effect in these strains, whereas levels of the same isotypes were increased significantly by pristane treatment in B6 controls.

Effects of pristane on survival and proteinuria

Pristane did not promote the development of renal disease in B6/lpr or B6/gld mice. Proteinuria ≥3+ was not seen in any of the mice (Table III), and only a single PBS-treated B6/lpr mouse developed ≥2+ proteinuria. Early death following pristane injection seldom occurs in BALB/c mice. However, 10–50% of mice with a B6 background died within 1 mo of pristane injection due to pulmonary hemorrhage of unknown cause (H. B. Richards, et al., unpublished data). In the present study, two B6 and three B6/gld mice died within 1 mo following pristane injection.

Late demise generally is not due to pulmonary hemorrhage. Two of 7 PBS-treated B6/lpr mice, 2 of 12 pristane-treated B6/lpr mice, 4 of 8 PBS-treated B6/gld mice, and 5 of 12 pristane-treated B6/gld mice died between 1 and 6 mo. However, there was no statistical difference in mortality rates of PBS- vs pristane-treated mice (p ≥ 0.04, Mann-Whitney, Table IV). In contrast, in pristane-treated B6/lpr mice, the weights of liver (p = 0.028), spleen (p = 0.04), and possibly lymph nodes (p = 0.075) all were less than that of PBS-treated controls, suggesting that pristane did not enhance lymphoproliferation in B6/lpr mice, but rather, suppressed it. This trend was not apparent in B6/gld mice, although the weight of the thymus (plus mediastinal lymph nodes) of pristane-treated mice was greater than that of PBS-treated mice. The latter did not reach statistical significance, however.

Cytokine production

In view of the evidence that cytokines play a role in the pathogenesis of autoantibodies in pristane-induced lupus (12) (H. B. Richards, manuscript in preparation), the levels of IL-6, IL-10, IL-12, and TNF-α in peritoneal lavage were examined (Fig. 6). Spontaneous production of all four cytokines was minimal in B6 mice (PBS treated). In contrast, B6/lpr mice spontaneously produced high levels of IL-12, and some also produced TNF-α (Fig. 6, C and D). Pristane treatment induced the production of IL-12 and smaller amounts of TNF-α, but not IL-6 in B6 mice. It also induced the production of IL-6 (p = 0.0193) and TNF-α in B6/lpr mice. In contrast to B6 controls, IL-12 production was unchanged in B6/lpr mice by pristane treatment.

Neither IL-4 nor IFN-γ was detectable in significant amounts in the peritoneal lavage fluid from PBS- or pristane-treated B6 wild type, lpr, gld, or lpr/gld mice (data not shown). As a surrogate marker of Th1 vs Th2 cytokine balance, the ratio of total IgG2a to IgG1 was evaluated 5 mo after treatment. As shown in Fig. 7, PBS-as well as pristane-treated B6 (+/+) mice had a significantly higher ratio than B6/lpr or B6/gld (for pristane-treated mice, p = 0.003 vs B6/lpr and p = 0.0027 vs B6/gld by Mann-Whitney). These data suggest that Fas or Fas ligand deficiencies increase serum levels of IgG1 (IL-4 dependent) out of proportion to IgG2a (IFN-γ dependent), despite increased spontaneous production of IL-12 in the peritoneal cavity (Fig. 6). However, this may be unique to the B6 background, in view of the marked IgG2a hypergammaglobulinemia in MRL/lpr mice.

Discussion

The lpr and gld defects accelerate the development of lupus-like disease in MRL mice (5–7). We examined the possibility that these defects also might act synergistically with an exogenous inflammatory stimulus (pristane) that induces lupus. Unexpectedly, defective Fas signaling and pristane exposure were mutually antagonistic. Pristane failed to enhance spontaneous production of anti-DNA/chromatin autoantibodies in B6/lpr and B6/gld mice, whereas the lpr and gld mutations suppressed the ability of pristane to induce anti-nRNP/Sm

Table III. Frequency of proteinuria and mortality in B6 mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>n</th>
<th>Proteinuria ≥ 3+ (%)</th>
<th>Death &lt; 1 mo (%)</th>
<th>Death ≥ 1 mo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>PBS</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>+/+</td>
<td>Pristane</td>
<td>12</td>
<td>0</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>lpr/lpr</td>
<td>PBS</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>lpr/lpr</td>
<td>Pristane</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>gld/gld</td>
<td>PBS</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>gld/gld</td>
<td>Pristane</td>
<td>12</td>
<td>0</td>
<td>25</td>
<td>42</td>
</tr>
</tbody>
</table>

p ≤ 0.028, spleen (p = 0.04), and possibly lymph nodes (p = 0.075) all were less than that of PBS-treated controls, suggesting that pristane did not enhance lymphoproliferation in B6/lpr mice, but rather, suppressed it. This trend was not apparent in B6/gld mice, although the weight of the thymus (plus mediastinal lymph nodes) of pristane-treated mice was greater than that of PBS-treated mice. The latter did not reach statistical significance, however.

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Table IV. Organ weights of B6 mice 6 mo after PBS or pristane treatment

<table>
<thead>
<tr>
<th>Strain</th>
<th>Rx</th>
<th>n</th>
<th>Weight (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>+/+</td>
<td>PBS</td>
<td>6</td>
<td>1.18 ± 0.10</td>
</tr>
<tr>
<td>+/+</td>
<td>Pristane</td>
<td>6</td>
<td>1.36 ± 0.14</td>
</tr>
<tr>
<td>lpr/lpr</td>
<td>PBS</td>
<td>5</td>
<td>1.78 ± 0.21</td>
</tr>
<tr>
<td>lpr/lpr</td>
<td>Pristane</td>
<td>10</td>
<td>1.44 ± 0.25</td>
</tr>
<tr>
<td>gld/gld</td>
<td>PBS</td>
<td>4</td>
<td>1.90 ± 0.13</td>
</tr>
<tr>
<td>gld/gld</td>
<td>Pristane</td>
<td>4</td>
<td>2.01 ± 0.22</td>
</tr>
</tbody>
</table>

† Lymph node weight is the sum of cervical, axillary, paraaortic, and inguinal nodes. Thymus weight includes mediastinal lymph nodes. ND, not determined (organs too small to isolate reliably).

‡ The p values are for Mann-Whitney test, comparing PBS vs pristane-treated group in each strain.

NS, not significant.
and Su autoantibodies in B6 mice. We conclude that pristane exposure and defective Fas signaling act on different pathways of autoantibody formation.

**B6/lpr and B6/gld mice are resistant to autoantibody induction by pristane**

In contrast to the severe lupus in MRL/lpr mice or pristane-treated normal mice (2, 4, 5, 12), B6/lpr mice have an attenuated lupus-like syndrome characterized by the development of anti-chromatin/DNA Abs, but not anti-nRNP/Sm, anti-Su, or anti-ribosomal P Abs, nephritis, or arthritis (5). The restricted spectrum of autoantibodies in B6/lpr mice has been attributed to the absence of critical susceptibility genes. However, the production of anti-nRNP/Sm, anti-Su, and anti-ribosomal P autoantibodies by pristane-treated mice demonstrates conclusively that B6 mice are capable of responding to these Ags. The lpr defect apparently cannot turn on these autoantibodies in B6 mice despite accelerating their spontaneous production on the MRL background. Remarkably, the effect of pristane on autoantibody production was diametrically opposed to that of lpr or gld. Whereas B6 mice treated with pristane produced IgG anti-nRNP/Sm, anti-Su, and anti-ribosomal P, they did not produce anti-ssDNA or anti-chromatin Abs. Conversely, all B6/lpr and B6/gld mice spontaneously developed high levels of IgG anti-DNA/chromatin, but not anti-nRNP/Sm, anti-Su, or anti-ribosomal P. This reciprocal relationship is consistent with the existence of multiple pathways of autoantibody formation.

It is not simple to differentiate whether the effects of pristane and the lpr or gld defects are antagonistic vs the alternative possibility that 3-mo-old B6/lpr and B6/gld mice were preprogrammed to develop a particular autoantibody response and pristane merely failed to modify its course. However, we recently have obtained evidence that this resistance to pristane-induced lupus is a unique characteristic of lpr and gld mice. When pristane was given to NZB/W F1 and BXSB mice with preexisting signs of lupus-like autoimmunity, disease and autoantibody production were enhanced and accelerated (4). More importantly, the same thing was seen in MRL+/+ mice, but not in MRL/lpr mice (M. Satoh et al., manuscript in preparation). Thus, while we cannot completely exclude the possibility that the lpr and gld mice were preprogrammed at 3 mo, the possibility that the lpr or gld defects and pristane are mutually antagonistic may be more likely.

Fas-mediated apoptosis is thought to protect against autoimmunity by facilitating peripheral deletion of autoreactive T (17–19) and B (20–22) lymphocytes. Also, apoptotic cell death may avoid potentially harmful inflammatory effects following the release of necrotic cell constituents (7, 23, 24). However, the present observations are difficult to reconcile with this hypothesis, although we cannot exclude an abnormality in the deletion of autoreactive cells responsible for the spontaneous production of anti-DNA/chromatin in B6/lpr mice. Our data are more consistent with the possibility that Fas-mediated apoptosis enhances the processing and presentation in an immunogenic form of a subset of self Ags. This model receives support from the clustering and specific cleavage of multiple lupus autoantigens in surface blebs in apoptotic cells (25).

An alternative, but not mutually exclusive, hypothesis is that the nonspecific inflammatory response caused by pristane is a critical factor.
Pristane-treated BALB/cAn mice produce IgG anti-DNA/chromatin antibodies in B6 mice (Table III), nor did it enhance lymphoproliferation. Indeed, pristane treatment decreased spleen, liver, and lymph node weights of B6/lpr mice (Table IV). Pristane and LPS also had different effects on natural autoantibody production. LPS induces polyclonal B cell activation and anti-ssDNA Ab production in both normal (30) and B6/lpr mice (28). These were absent in pristane-treated B6/lpr and B6/gld mice (Fig. 4), but could be induced in Fas/Fas ligand-intact animals (4). We conclude that the effects of pristane in lpr or gld mice differ fundamentally from those of LPS.

Anti-DNA/chromatin response in B6 mice

Pristane-treated BALB/cAn mice produce IgG anti-DNA/chromatin autoantibodies at high levels in an IL-6- and IFN-γ-dependent manner (12). (H. B. Richards, et al., in preparation). The inability of pristane to induce these autoantibodies in B6 mice (Fig. 1) does not reflect unresponsiveness to chromatin because they are produced spontaneously at high levels in B6/lpr and B6/gld mice. Intraperitoneal injection of pristane led to local production of IL-12, but not IL-6, in the peritoneal cavity of B6 mice (Fig. 6, A and C). In contrast, pristane-treated BALB/c mice produce large amounts of IL-6 and IL-12 (V. Shaheen, et al., unpublished data), raising the possibility that both cytokines are needed to generate an anti-DNA/chromatin response in Fas/Fas ligand-intact mice. B6 mice may fail to generate an anti-DNA/chromatin following pristane treatment due to a defect in IL-6 production.

The cytokine requirements for anti-DNA/chromatin Abs may be different in mice with defective Fas signaling. The levels of IL-6 in peritoneal lavage from B6/lpr mice increased markedly following pristane treatment (Fig. 6A) even though autoantibody levels were not augmented (Fig. 1). It is unlikely, therefore, that IL-6 plays a major role in the production of anti-DNA/chromatin autoantibodies in B6/lpr mice, despite its importance in pristane-treated BALB/cAn mice (12).

The role of IL-12 in anti-DNA/chromatin autoantibody production by Fas-defective mice is less clear. IL-12 (31) and IFN-γ (32–34) are important for the production of anti-DNA Abs in MRL/lpr mice. However, we found no correlation between the level of IL-12 in the peritoneal lavage and the level of serum anti-DNA/chromatin (data not shown). The low IgG2a/IgG1 ratio in B6/lpr and B6/gld mice compared with B6 (Fig. 7) suggests that anti-DNA/chromatin Abs are produced in Fas-defective mice via an IL-4-dependent pathway (35). Paradoxically, however, B6/lpr mice spontaneously produced IL-12.

Spontaneous IL-12 production in B6/lpr mice

The spontaneous IL-12 production by B6/lpr mice has not been reported previously. However, in comparison with MRL+/−/+ peritoneal macrophages from MRL/lpr mice exhibit greatly increased IL-12 production in response to LPS plus IFN-γ (31). This could reflect acceleration of the autoimmune disease or could be a direct consequence of the lpr mutation. The latter possibility is supported by the enhanced spontaneous production of IL-12 in lpr mice (Fig. 6). The normal microbial flora of the intestine stimulates B and T lymphocytes as well as APCs in the peritoneal cavity (36–38). The elevated IL-12 levels in B6/lpr mice may reflect an inability to delete activated peritoneal APCs, because normal macrophages activated by IFN-γ and TNF-α up-regulate Fas expression and are eliminated by apoptosis (39, 40). Consistent with that notion, the macrophage compartment expands in MRL/lpr mice (41). Interestingly, the increased IL-12 production in B6/lpr mice does not lead to an increased ratio of IgG2a/IgG1, a surrogate marker for Th1/Th2 cytokine balance. Moreover, for unclear reasons, pristane appears to have only a small effect on the IgG2a/IgG1 ratio in B6 background mice at 5 mo. This strain is genetically skewed toward Th1 responses (42). In contrast, the IgG2a/IgG1 ratio increases dramatically in BALB/c (genetically skewed toward Th2 responses) and most other strains (M. Satoh, unpublished data). However, despite these differences, both BALB/c and B6 mice are susceptible to the induction of anti-nRNP/Sm and Su autoantibodies.

In summary, the lpr and gld mutations may either accelerate or retard the development of autoimmunity, possibly reflecting the existence of different pathways of autoantibody formation. Further defining these pathways may pave the way to a better understanding of the variable phenotype of human SLE.

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References


