B and T cell tolerance and autoimmunity in autoantibody transgenic mice

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Abstract

One hallmark of systemic lupus erythematosus (SLE) is the presence of autoantibodies directed against a diverse group of proteins of the U1/Sm small nuclear ribonucleoprotein particles (snRNP). Patients with SLE and murine models of this disease generate high titers of affinity mature, isotype-switched autoantibodies characteristic of T cell-dependent immune responses. In this investigation, we made use of anti-snRNP Ig transgenic mice (2-12 Tg) to track regulation of autoreactive B cells in normal and autoimmune-prone mice. Autoantibody studies demonstrated that the regulation of anti-snRNP B cells is intact in non-autoimmune Tg mice, but not in MRL-1pr/lpr mice. We further utilize autoreactive Tg B cells as antigen-presenting cells (APC) and individual snRNP peptides to assess the presence of autoreactive T cells in the repertoire of non-autoimmune and MRL-1pr/lpr mice. We found that Tg B cells can direct specific T cell tolerance in a non-autoimmune-prone (C57Bl/6) background, whereas the same autoantibody transgene in MRL-1pr/lpr mice drives T cell autoimmunity. Moreover, Tg B cell APC could stimulate autoreactive T cells from wild-type (non-Tg) C57Bl/6 mice, indicating a lack of tolerance induction in the absence of the autoantigenic-presenting B cells. Thus, we have defined dual roles for autoantigen-presenting B lymphocytes in stimulating self-reactive T cells that inhabit the normal repertoire or, under some conditions, providing tolerance signals.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of autoantibodies to a number of common cytoplasmic and nuclear components (1). One important target of autoimmune responses in human SLE and in murine models of the disease is a group of small nuclear ribonucleoprotein particles (snRNP) designated U1 and Sm (2–4). Patients with SLE have autoantibodies that are directed at a diverse array of determinants on the 70-kDa, A and C protein components of the U1 snRNP and the B/B' and D protein of the Sm snRNP. These autoantibody responses are typical of other antigen-driven immune responses in their isotype expression, their degree of somatic mutation and their dependence on CD4 T cell responses (5–8).

In addition to their functions in antibody synthesis, B cells are also effective antigen-presenting cells (APC). Surface Ig receptor uptake of specific antigen can stimulate T lymphocytes nearly 10,000-fold more efficiently than non-specific APC (9,10). We and others have previously found that this property has important implications in the induction of autoimmune responses (11–16). B cells with the ability to concentrate self-antigens may activate T cells that are in low numbers or have low affinity for self-peptide in the periphery. It is already clear that the threshold of self-antigen and/or affinity for self-peptides helps define central T cell development in the thymus. Moreover, support for an active role of B cells in lupus pathogenesis has been elucidated by the analysis of B cell-deficient (JhD) MRL-1pr/lpr mice (15,16). B cell-deficient MRL-1pr/lpr mice do not develop populations of activated T cells as are found spontaneously in wild-type mice. The reconstitution of JhD MRL-1pr/lpr mice with B cells that do not secrete Ig reconstitutes T cell activation in a manner comparable to wild-type MRL-1pr/lpr mice. These studies suggest that B cells play a central role in the promotion of systemic autoimmunity by the direct activation of autoreactive CD4 T cells through the presentation of autoantigenic peptides.

As demonstrated by other investigators, B cells may also have a role in the induction of peripheral T cell tolerance (17–21). These seemingly contradictory roles for B cells have been difficult to fully resolve. In vitro and in vivo studies using resting or previously activated populations of T and B cells suggest...
that the outcomes of this cognate interaction may be dependent on a number of factors, including the activation status of both T and B cells during their initial encounter (22,23). Our own prior studies implicate a requirement for surface B7–CD28 interactions when B cells prime autoreactive T cells in normal mouse strains (24). However, autoimmune-prone MRL-lpr/lpr mice develop activated T cells, high titers of autoantibodies and pathology in the complete absence of B7-1 and B7-2 (25,26). In either case, whether in the induction of T cell tolerance or activation, self-reactive B cells must survive selective pressures of their own to perform these functions. Studies using Ig transgenes directed towards the model antigen hen egg lysozyme, MHC class I or the double-stranded DNA autoantigen of lupus have observed several developmental outcomes for autoreactive B cells in non-autoimmune mice. Among these transgenic (Tg) models, self-reactive B cells may experience clonal deletion, anergy or receptor editing and may be receptive to stimulation with self-antigen (27–36). A model utilizing rheumatoid factor Ig transgenes has demonstrated immunologic ‘ignorance’ that could be overcome in the presence of the stimulating autoantigen (37).

In the present study, we have utilized autoantibody Tg mice on both normal and autoimmune-prone murine backgrounds in attempts to identify features that may dictate the induction of T cell tolerance versus autoimmunity. Anti-snRNP Ig μ chain Tg (2-12 Tg) mice have been developed previously using an unmutated, rearranged VhJ558 gene derived from an MRL-lpr/lpr anti-Sm hybridoma designated 2-12 (32). The 2-12 Ig transgene supports B cell development with about one-third of 

**Methods**

**Ig Tg mice**

Anti-snRNP B cell hybridoma, designated 2-12, was originally cloned from an MRL-lpr/lpr mouse (38). Ig Tg mice were derived using the rearranged VDJ segment of the 2-12 anti-snRNP hybridoma cloned upstream in a vector containing the Cμ region gene segment. Tg founders were backcrossed for >10 generations to B10.A, C57BL/6 mice or MRL-lpr/lpr mice. Presence of the transgene was identified by PCR analysis of tail DNA using 2-12-specific primers 5’-GAGGTCCAGCTG-CAGCAGTCTGGGA-3’ in the first coding region of the V region and 5’-CGCTCCACCAGCCTCTAGA-3’ complementary to the XbaI site downstream of JH4. Animals were age and sex matched in all experiments, and housed in a conventional facility at Yale University.

**Indirect immunofluorescence (antinuclear antibodies)**

Indirect immunofluorescence assays were performed using commercially available cell substrates (Inova, San Diego, CA). In brief, 40 μl of a 1:40 dilution of serum was placed on slides coated with human epithelial cells (HEp-2) and incubated at room temperature for 2 h. After a 5-min wash in PBS, FITC-conjugated anti-mouse IgM or IgG (Sigma, St Louis, MO) was applied to individual wells and incubated in the dark at room temperature for 2 h. After another 5-min wash, wells were examined by UV-fluorescence microscopy.

**Measurement of anti-snRNP response by ELISA**

Detection of anti-snRNP by ELISA was performed as previously described (25). Briefly, 96-well PVC plates were coated with native snRNP antigen in carbonate-buffered saline, washed and blocked with PBS/BSA/Tween. Mouse sera were diluted at 1:100 in PBS and then added to antigen-coated plates in duplicate. Anti-mouse IgM–alkaline phosphatase (Southern Biotechnology Associates, Birmingham, AL) was used as secondary antibody and diluted at 1:1000. Assays were developed with 1 mg/ml p-nitrophenylphosphate (Sigma) and the OD405 determined at various timepoints. Experimental values from separate experiments were normalized to a single MRL-lpr/lpr positive control serum used in every assay (arbitrarily defined as 100 U).

**Kidney pathology**

Kidneys from Tg or wild-type mice were collected at the indicated timepoints and immediately immersed in 10% formalin (Fisher, Pittsburgh, PA). Thin sections and H & E staining were performed by Yale Dermatopathology Laboratory. Blinded samples were examined for pathology at ×100 magnification.

**Lymphocyte proliferation assays**

Conventional T lymphocyte proliferation assays were performed with purified CD4 T cells (2–3 × 10^6 cells/ml) and irradiated purified B cells (500 rad) as APC (8–12 × 10^5 cells/ml). All assays were set up with triplicate samples and incubated with antigen titrations for 4 days. Lymphocyte proliferation was assessed by [³H]thymidine incorporation (1.0 μCi/well; ICN, Irvine, CA) during the last 18 h of culture. Sample wells were harvested onto filters and incorporated radioactivity was counted in a Betaplate liquid scintillation counter (LKB/Wallac, Gaithersburg, MD).

CD4 T cells were purified using a two-step protocol. Unfractionated spleen cells were incubated with a cocktail of antibodies to CD8 (TIB208), B cells (TIB146), monocytes/macrophages (TIB128) and anti-class II antibodies (Y3JP) at 4°C for 1 h. Excess antibodies were washed off followed by incubation with sheep anti-mouse/rat Ig-coated magnetic beads (Perseptive Biosystems, Cambridge, MA) at a ratio of 5:1 (beads:cells) for 1 h at 4°C. The unbound CD4 cells were separated from other cell populations on a magnet and the
beads. In brief, lymphocytes from splenocytes were enriched cloned by PCR from a murine cDNA library. The snRNP D C57B1/6 wild-type and 2-12 Tg mice B cell purification (Miltenyi Biotech, Auburn, CA) and passed through a MACS separator. The purity of B cells was >95% as assessed by flow cytometry.

unbound cells were then subjected to a second round of purification as described above. The purity of CD4 cells was >96% as assessed by flow cytometry.

B cell purification

B cells were purified using negative selection and magnetic beads. In brief, lymphocytes from splenocytes were enriched by Ficoll-Hypaque (Sigma) density separation. Cells were labeled with anti-mouse CD43 and anti-mouse CD90 microbeads (Miltenyi Biotec, Auburn, CA) and passed through a separation column that is placed in the magnetic field of a MACS separator. The purity of B cells was >95% as assessed by flow cytometry.

Generation and analysis of T cell hybridomas

T cell hybridomas were derived from unmanipulated MRL-lpr/lpr 2-12 Tg mice. These mice were sacrificed and splenocytes were fused with the BW 5147 T cell thymoma cell line to generate T cell hybridomas. T cell hybridomas that stained positive for the TCR were used for further screening. The T cell hybridomas were then assayed for specificity using APC from 2-12 Tg mice and pools of snRNP D peptides (Yale University, Keck Laboratory of Protein Synthesis) as sources of antigen. All assays were set up in triplicate and supernatants from these cultures were harvested 24 h later and assayed for IL-2 production using the CTLL cell line. CTLL cell proliferation was assessed by [3H]thymidine incorporation as described above. Hybridomas that responded to snRNP D peptides presented by 2-12 Tg APC were used in subsequent assays.

Antigens and immunizations

Synthetic peptides of the D protein of the murine snRNP and recombinant murine D (rD) protein expressed in Escherichia coli were used as sources of antigen in T cell proliferation assays. A total of 22 peptides (15mers) of the murine snRNP D protein were synthesized for the analysis of T cell specificity. Peptides overlapped by five amino acids along the entire length of the D protein. To express rD protein, the cDNA encoding the open reading frame of the snRNP D gene was cloned by PCR from a murine cDNA library. The snRNP D cDNA was subcloned into the vector pBluescript II KS (Stratagene, La Jolla, CA). Sequencing by the Sanger dideoxynucleotide method confirmed that the 372-bp cDNA was identical to that of the published sequence of murine snRNP D (39). rD was expressed in induced E. coli and purified by anion-exchange chromatography as described previously (39). rD protein was absorbed for potential mitogens by anti-lipopolysaccharide column chromatography using agarose beads coated with PolyImyn B. Part of the rD preparation was labeled with biotin for FACS staining or tissue section staining.

In some experiments, wild-type or Tg C57Bl/6 mice were immunized with rD protein followed by examination of serum antibodies by ELISA. For these studies, groups of five wild-type or Tg C57Bl/6 mice were immunized with 50 µg of purified rD emulsified in complete Freund’s adjuvant (CFA) in the hind footpad and in the base of the tail. Mice were boosted with the same amount of rD in incomplete Freund’s adjuvant at day 21 and antibody assays were performed at day 28. For the analysis of serum autoantibodies, conventional ELISAs were performed utilizing native murine snRNP purified from Ehrlich ascites cultured cells as previously described (39).

 Immunoprecipitation and immunoblotting

Immunoprecipitation assays for intracellular phosphorylation were performed as previously described (40). Ten million purified B cells as indicated above were incubated for 5 min at 37°C with anti-IgM (10 µg/ml) or rSm-D self-antigen (5 µg/ml) of RPMI 1640 without FCS. The cells were pelleted by centrifugation and lysed in 200 µl of RIPA buffer supplemented with 1 mM PMSF, 1 mM leupeptin, 100 mM NaF and 1 mM sodium orthovanadate. For the detection of Syk kinase phosphorylation, supernatants were incubated with 1 µg rabbit anti-Syk (C-20; Santa Cruz Biotechnology, Santa Cruz, CA) and 40 µl of Protein A/G-Sepharose for 90 min at 4°C. The Sepharose beads were pelleted, washed 3 times with RIPA buffer, suspended in SDS sample buffer and boiled for 5 min. The immunoprecipitates were analyzed on SDS-PAGE gel. Proteins were transferred onto nitrocellulose membranes and blotted with anti-phosphotyrosine antibody or anti-Syk antibody (Santa Cruz Biotechnology). In separate studies, whole-cell lysates were probed for tyrosine phosphorylation. In brief, whole-cell lysates were resolved by 7.5% SDS–PAGE gel and blotted with anti-phosphotyrosine antibody (Santa Cruz Biotechnology) essentially as described (40). The immunoreactive antibody was detected using the enhanced chemiluminescence system (Cell Signaling Technology, Beverly, MA).

Results

Anti-snRNP B cells are developmentally arrested in normal 2-12 Tg mice, but are activated in MRL-lpr/lpr mice

As demonstrated in earlier studies, the 2-12 heavy chain transgene supports B cell development, but the majority of anti-snRNP B cells are of immature phenotype (32). Overall, nearly 30% of Tg B cells are autoreactive, binding either snRNP or single-stranded DNA by flow cytometry (32,41). To investigate whether anti-snRNP B cells are activated and make autoantibodies, serum samples from Tg mice of different backgrounds were collected at regular intervals and autoantibody production was examined by ELISA. As indicated in

Table 1. Anti-snRNP (IgM) response in MRL-lpr, B10.A, C57Bl/6 wild-type and 2-12 Tg mice

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Age (weeks)</th>
<th>n</th>
<th>Anti-snRNP (IgM, U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10.A</td>
<td>14–16</td>
<td>10</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>B10.A 2-12 Tg</td>
<td>16</td>
<td>12</td>
<td>27 ± 5 P3</td>
</tr>
<tr>
<td>C57Bl/6</td>
<td>14–16</td>
<td>14</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>C57Bl/6 2-12 Tg</td>
<td>16</td>
<td>14</td>
<td>28 ± 8 P3</td>
</tr>
<tr>
<td>MRL-lpr/lpr 2-12 Tg</td>
<td>16–18</td>
<td>20</td>
<td>109 ± 19 P1</td>
</tr>
</tbody>
</table>

aSera (1:100 dilution) were tested at the same time levels of anti-snRNP (IgM) using ELISA as described in Methods.

bThe OD values of one MRL-lpr mouse serum (as a standard used in all ELISA assays) were arbitrarily defined as 100 U. The units in different mice were obtained by the formula ODexp/ODstd × 100 (U).

cP <0.001 for comparisons to wild-type B10.A mice; P2 >0.05 for comparisons to the corresponding wild-type mice.

T-B cell regulation in autoantibody transgenic mice
Fig. 1. Antinuclear autoantibodies arise spontaneously in Tg MRL--lpr/lpr mice in contrast to Tg non-autoimmune-prone mice. Serum dilutions from 2-12 Tg or wild-type mice, as indicated, were examined by indirect immunofluorescence on Hep-2 cell substrates. Panels are representative fluorescence patterns of age-matched mice.

Fig. 2. Kidney pathology of wild-type or 2-12 Tg B10.A, C57Bl/6, MRL-lpr/lpr mice. Original magnification: ×100.
Table 1, Tg mice in B10.A and C57Bl/6 backgrounds could not make detectable anti-snRNP autoantibodies above levels found in the corresponding wild-type mouse strains ($P_1 > 0.05$). In contrast, Tg MRL-lpr/lpr mice spontaneously produce elevated amounts of anti-snRNP autoantibodies compared to wild-type B10.A mice ($P_1 < 0.001$). Antinuclear antibody assay also indicates that Tg B cells are activated in MRL-lpr/lpr, but not in the non-autoimmune-prone mouse (Fig. 1). The presence of autoantibody production indicates that the regulation of anti-snRNP B cell tolerance is not intact in MRL-lpr/lpr Tg mice. This also implied that endogenous T cell help and a source of endogenous snRNP autoantigen contributed to the autoimmune response. Conversely, the absence of spontaneous autoantibody production in Tg B10.A and C57Bl/6 mice suggested that T cell tolerance to the self-antigen was maintained and/or no source of self-antigen was available to drive the autoimmune responses.

Kidney pathology

Glomerulonephritis is one pathologic hallmark of SLE. To determine whether the anti-snRNP transgene would cause kidney pathology, we evaluated B10.A, C57Bl/6 and MRL-Fas/lpr/lpr mice at various timepoints (2, 4 and 6 months of age). The examination of kidney sections from normal mice showed preservation of the renal architecture with normal vessels and glomeruli (Fig. 2A and C). B10.A or C57Bl/6 2-12 Tg mice show a similar pattern to those of normal wild-type mice (Fig. 2B and D).

The most striking aspects of the kidney pathology were found in MRL-lpr/lpr 2-12 Tg mice, where significant interstitial accumulations of lymphocytes (Fig. 2F) were prominent beginning at 16 weeks of age. However, there was no evidence of endovasculitis in MRL-2-12 Tg mice. Control MRL-lpr/lpr mice (>16 weeks old) demonstrated large perivascular mixed lymphoid hyperplasia which included activated lymphocytes, plasma cells and mononuclear cells typical of pathology found in these mice (Fig. 2E). The vessels showed marked myointimal proliferation and infiltration of the myointimal region with lymphocytes.

Anti-snRNP B cells tolerate autoreactive T cells in normal Tg mice but activate D-protein-responsive T cells in autoimmune-prone mice

The importance of B cells as APC has been studied in our laboratory and others (11,42). To understand whether anti-snRNP B cells can serve as autoantigen-presenting cells and to establish T cell tolerance or activation, we investigated the response of CD4 T cells from unimmunized C57BL/6 wild-type and Tg mice to the snRNP D protein in the presence of either wild-type or Tg APC. As described earlier, the Ig transgene was selected for its binding specificity to the D protein component of the murine snRNP complex. As illustrated in Fig. 3, CD4 T cells from wild-type C57Bl/6 mice did not respond to snRNP D protein presented by wild-type autologous APC. Surprisingly, wild-type C57Bl/6 CD4 T cells responded strongly to snRNP D protein presented by Tg APC (representative proliferative data from seven experiments), suggesting that unique peptides presented by Tg APC could activate autoimmune T cells from the normal repertoire of a non-autoimmune-prone mouse (Fig. 3A). This simple observation suggests that the Tg B cells, by virtue of their surface receptor specificity, presented a unique group of snRNP D peptides to which T cells in the wild-type mouse had never been exposed and thus had never been tolerized.

In contrast, CD4 T cells originating from Tg C57Bl/6 mice failed to respond significantly to snRNP peptide presented by wild-type APC or by syngeneic Tg APC. The inability of CD4 T cells originating from Ig Tg mice to respond under these conditions suggests that Tg B cells (or their secreted antibody), in vivo, acted to tolerate T cells to the specific peptides that were displayed on its surface.

As in C57Bl/6 mice, CD4 T cells from wild-type or 2-12 Tg MRL-lpr/lpr mice failed to respond to snRNP D protein presented by wild-type syngeneic APC (Fig. 3B). However, T cells from 2-12 Tg MRL-lpr/lpr proliferated strongly when exposed to peptides on Tg APC. MRL-lpr/lpr T cells, whether from a 2-12 Tg or wild-type source, require Tg APC to drive T cell proliferation. These results indicate that autoreactive T cells are not tolerized by Tg B cells in the MRL-lpr/lpr mouse, unlike the situation in non-autoimmune-prone mice.

snRNP D protein-reactive T cell hybridomas from MRL-lpr/lpr 2-12 Tg mice

To further confirm the specificity of T cell responses described above, we generated T cell hybridomas from unmanipulated anti-snRNP Tg MRL-lpr/lpr mice. Sixty hybridomas were screened by staining for TCR and reactivity to a pool of snRNP D peptides presented by 2-12 Tg APC. Fourteen T cell hybridoma clones were found to respond to one or more snRNP D peptides and Tg APC. One representative hybridoma, designated clone 26, was isolated and found to respond to the snRNP D peptide (31±45) presented by 2-12 Tg APC (Fig. 4). This observation formally demonstrates that snRNP-reactive T cells are not tolerized by the transgene-bearing B cells in MRL-lpr/lpr mice. In contrast, no snRNP-reactive T cell clones could by derived by similar methodology from Tg C57Bl/6 mice, further suggesting that tolerance was induced by endogenous anti-snRNP B cells (data not shown).

Immunization of C57Bl/6 Tg mice with snRNP D protein induces anti-snRNP antibody responses

We have demonstrated that all strains of 2-12 Tg mice have a significant number of anti-snRNP D protein B cells in the periphery. However, no circulating anti-snRNP autoantibodies were found in C57Bl/6 or B10.A non-autoimmune-prone Tg mice. In contrast, 2-12 Tg MRL-lpr/lpr mice spontaneously secrete high titers of autoantibody. Prior studies demonstrated that immunization with intact snRNP particles in CFA could elicit autoantibody synthesis in Tg C57Bl/6 mice (32). In the present studies we needed to confirm that rD protein accurately represents the native autoantigen. Wild-type and 2-12 Tg C57Bl/6 mice were immunized with rD protein in CFA, boosted with rD protein in IFA on day 21, and antibody responses were examined at day 28. As seen in Fig. 5, wild-type mice have no detectable autoantibodies after immunization with rD protein in a manner in which tolerance is observed after immunization with the native mouse snRNP particle (39). No T cell responses can be elicited in wild-type mice (data not shown), suggesting...
immune tolerance to this recombinant self-protein. However, immunization of Tg mice with rD in CFA elicited strong autoantibody responses. This outcome demonstrated that anti-snRNP B cells are not irreversibly anergic and that B cell tolerance can be broken in non-autoimmune strains of 2-12 Tg mice by supplying self-antigen in the context of CFA adjuvant.

Syk kinase is phosphorylated in B10.A anti-snRNP B cells following rSm-D stimulation while it is constitutively phosphorylated in MRL-lpr/lpr mice

Thus far, we have demonstrated the induction of activation of autoreactive T cells by anti-snRNP B cells of MRL-lpr/lpr Tg mice while induction of tolerance in normal background mice. It is possible that distinct phenotypes of anti-snRNP B cells exist in different strains of mice. It has been shown that Syk kinase phosphorylation is a marker of antigen-activated B cell development [reviewed in (43,44)]. As discussed above, we have indicated that most of anti-snRNP B cells in normal mice are of an immature phenotype (32). To determine if Syk phosphorylation is related to immature anti-snRNP B cell development, we purified B cells from Tg or wild-type B10.A mice followed by stimulation with anti-IgM or rSm-D self-antigen. As indicated in Fig. 6(A), anti-snRNP B cells were indistinguishable from resting Tg B cells in their ability to phosphorylate Syk upon anti-IgM stimulation (Fig. 6A, panel B). In contrast, anti-snRNP B cells exhibited Syk phosphorylation in response to rSm-D while no band was found in Tg mice (Fig. 6A, panel A). B cells from either Tg or wild-type MRL-lpr/lpr mice (16–30 weeks) showed Syk kinase phosphorylation even without antigen stimulation indicating that endogenous self-antigen or other factors serve to stimulate B cell maturation (Fig. 6A, panel C). It is not yet known if constitutive Syk phosphorylation in MRL-lpr/lpr mice is a function of Fas deficiency. In addition, overall tyrosine phosphorylation is enhanced in activated anti-snRNP Tg B cells (Fig. 6B). One band in particular was enhanced in lysates of Tg B cells (Fig. 6B, arrow).

Discussion

The immune system has evolved a number of sophisticated mechanisms in order to direct the response of lymphocytes against foreign antigens while ignoring self-proteins. However, the presence of autoimmune diseases suggests that a balance exists between tolerance versus immunity to self-proteins. SLE represents one disease for which a failure of tolerance occurs. Autoimmunity in SLE is directed against a number of intracellular macromolecules including nucleosomes, DNA and snRNP (1), leading to pathology in the kidneys, skin and connective tissues. A number of prior studies have indicated that fundamental defects in B cell and T cell functions may be responsible for the autoimmune responses of SLE (45–47).

We have utilized mice made Tg for the heavy chain of an anti-snRNP autoantibody in order to amplify or exaggerate the cognate interactions of autoreactive B cells and T cells in microenvironments in vivo. Investigations from the Clarke laboratory have demonstrated that these self-reactive B cells are not deleted or anergic in the repertoire of mice (32). Indeed, these B cells are responsive to stimulation with snRNP antigen to synthesize and secrete autoantibodies (32,48). The present study has examined the role of anti-snRNP B cells in providing tolerance and/or activating signals to autoreactive T cells.

Three important observations have arisen from these studies. Among the most important is the finding that autoreactive T cells inhabit the naive repertoire of normal mice (Fig. 3). Second, a lack of both B and T cell tolerance is found in Tg MRL mice. In contrast, no spontaneous autoimmunity is found when the same transgene is placed in non-autoimmune-prone mice. T cell tolerance is induced in Tg C57Bl/6 or Tg B10.A mice. Finally, autoreactive B cells can find sources of snRNP autoantigens in both MRL and normal mice for uptake and presentation to T lymphocytes.

Among these observations, it was surprising to find that many snRNP-reactive T cells escape thymic and peripheral tolerance mechanisms in normal mice. We used Tg B cells as probes to identify and activate autoreactive T cells in the
repertoire of wild-type C57B1/6 mice (Fig. 3). Even in non-autoimmune mice, snRNP-reactive T cells have evaded negative selection in the thymus, tolerance induction in the periphery and have clearly not died by neglect. We hypothesize that T cells have avoided peripheral tolerance to some specific snRNP peptides in normal mice because they have never come in contact with Tg B cells presenting snRNP peptide. This assumption is supported by prior work from our laboratories demonstrating a lack of T cell tolerance to several synthetic peptides of the snRNP D protein when used as immunogens in normal strains of mice (39). Here, however, we show responses to whole protein naturally processed by B cells. It is interesting to note that the immunodominant peptide found to activate T cell clones in the present work overlaps with a cryptic peptide immunogen found in our earlier studies (39). We had originally found that autoantibodies and autoreactive T cells could be induced in mice by immunization with p26-40 of the snRNP D protein. T cell clones from unimmunized Tg mice analyzed in this study actively respond to an overlapping peptide at this site, p31-45 (Fig. 4). While we have not identified peptide sequences from the MHC class II cleft of Tg B cells, we instead utilized synthetic peptides to identify the ensuing autoreactive T cell specificities as an indirect reflection of the peptides presented by Tg B cells.

In observations similar to those we have reported here, autoreactive T cells have previously been identified from the naive repertoire of mice and from humans without clinical autoimmune disease (49, 50). Recent studies by Anderson et al. have found autoreactive T cells specific for a target peptide of experimental autoimmune encephalomyelitis in the naive lymphoid repertoire of all H-2^d strains of mice (49). One target peptide of myelin proteolipid protein (PLP) was found to stimulate T cells from naive SJL, BALB/s and B10.S mice suggesting a failure of thymic negative selection for this autoantigen. T cells specific for other target autoantigens of multiple sclerosis including myelin basic protein, PLP and myelin oligodendrocyte glycoprotein have been found in the peripheral repertoire of normal humans (50). From studies with TCR Tg animals, it is now clear that a large percentage of autoreactive T cells can escape negative selection in the thymus even in the presence of the deleting peptide ligand (51). The logical question arises as to why a species with autoreactive T cells specific for lupus autoantigens or to experimental autoimmune encephalomyelitis antigens fails to develop spontaneous autoimmune disease. It is possible that...
these T cells either fail to develop into effector phenotypes or are subject to other peripheral regulatory T cells. Studies are underway in our laboratory to determine if Tg B cells that drive autoactive T cell responses in MRL mice can provide the same T cell stimulus from the repertoire of MHC matched non-autoimmune strains of mice.

A limited autoreactive TCR specificity was apparent from our analysis of hybrid clones derived from Tg MRL-lpr/lpr mice (Fig. 4 and data not shown). This is most likely due to the fact that surface Ig specificity of antigen-presenting B cells influences the internal processing of proteins and shapes the repertoire of T cell responses. Several studies have demonstrated that the fine specificity of surface Ig controls the patterns of antigen fragmentation and the subsequent repertoire of responding T cells (52–54). Moreover, the avidity of slg binding to its ligand directly affects the efficiency of antigen presentation by B cells.

We have demonstrated that autoreactive B cells can be stimulated to secrete autoantibodies by providing self-antigen with CFA adjuvant. The co-stimulatory molecules B7-1 and B7-2 are known to play a pivotal role in determining the recognition of antigen by T cells and could lead to activation or anergy depending on their presence or absence. However, it does not appear that either B7-1 or B7-2 expression differences between non-autoimmune strains versus MRL-lpr/lpr reflect differences in T cell tolerance or activation (25,26). We do not yet know whether other potential co-stimulation ligand/receptor such as B7n/ICOS interactions may explain the differences in cognate B–T cell interactions in these mice although these investigations are underway.

Although it is still not clear why anti-snRNP B cells have differential roles in the induction of activation/tolerance of autoreactive T cells, our data suggest that it might be related to the different status of anti-snRNP B cells. Syk kinase has been demonstrated to play a crucial role in B cell activation and development (44). Additionally, Syk kinase and BCR receptor both influence BCR-mediated MHC class II-restricted antigen presentation (55). Immunoprecipitation studies suggest that Syk kinase is constitutively phosphorylated in MRL-lpr/lpr mice, indicating anti-snRNP B cells are by default in activation, presumably through circulating self-antigens. Anti-snRNP B cells in the normal background mice have the same activation, presumably through circulating self-antigens. Anti-lpr that the source or affinity of endogenous antigen for BCR is not autoreactive T cell responses in MRL mice can provide the gest that Syk kinase is constitutively phosphorylated in MRL-autoimmune strains versus MRL-autoimmune strains of mice.

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