Regulation of Anti–double-stranded DNA B Cells in Nonautoimmune Mice: Localization to the T–B Interface of the Splenic Follicle

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Summary

Systemic lupus erythematosus (SLE) and the MRL-lpr/lpr murine model for SLE are characterized by the presence of serum anti–double-stranded (ds)DNA antibodies (Abs), whereas nonautoimmune individuals have negligible levels of these Abs. To increase the frequency of anti-DNA B cells and identify the mechanisms involved in their regulation in nonautoimmune mice, we have used Ig transgenes (tg). In the present study, we used the VH3H9 heavy (H) chain tg which expresses an H chain that was repeatedly isolated from anti-dsDNA Abs from MRL-lpr/lpr mice. Because the VH3H9 H chain can pair with endogenous L chains to generate anti–single-stranded DNA, anti-dsDNA, and non-DNA B cells, this allowed us to study the regulation of anti-dsDNA B cells in the context of a diverse B cell repertoire. We have identified anti-dsDNA B cells that are located at the T–B interface in the splenic follicle where they have an increased in vivo turnover rate. These anti-dsDNA B cells exhibit a unique surface phenotype suggesting developmental arrest due to antigen exposure.

Ig transgenic models to neo-self Ags have helped to classify two manifestations of B cell tolerance: clonal deletion and functional inactivation (anergy) (1–3). Recently, the distinction between these two has come into question as “anergized” cells have been shown to have a reduced lifespan and may be in a state of “delayed deletion” (4). Furthermore, the relative contribution of deletion versus receptor editing to the elimination of autoreactive B cells is being reevaluated (5, 6). Given that most autoimmune diseases are characterized by the presence of autoantibodies directed toward a discrete set of autoantigens, we are interested in determining whether the mechanisms described for the maintenance of tolerance to neo-self Ags apply to disease-associated autoantigens.

Anti–double-stranded (ds) DNA Abs are one of the hallmarks of SLE and the MRL-lpr/lpr murine model for SLE, and rising titers of these Abs correlate with disease exacerbation (7). In the serum of nonautoimmune individuals, anti-dsDNA Abs are not present, suggesting that this specificity is regulated, yet the mechanism governing this regulation remains unclear. To follow the fate of anti-dsDNA B cells, we have used Ig transgenic mice. The transgene (tg) being studied encodes the VH3H9 H chain, originally isolated from anti-dsDNA Igs in diseased MRL-lpr/lpr mice, in combination with different L chains (8). Transfection studies have shown that this H chain can pair with a variety of different L chains to generate both anti–single-stranded (ss)DNA and anti-dsDNA Abs (9). As a tg, VH3H9 can pair with endogenous L chains to generate anti-ssDNA, anti-dsDNA, and non-DNA B cells, allowing us to study the regulation of anti-dsDNA B cells in the context of a diverse B cell repertoire. We have identified anti-dsDNA B cells that are located at the T–B interface in the splenic follicle where they have an increased in vivo turnover rate. These anti-dsDNA B cells exhibit a unique surface phenotype suggesting developmental arrest due to antigen exposure.

1 Abbreviations used in this paper: AP, alkaline phosphatase; BrdU, bromodeoxyuridine; ds, double-stranded; HEL, hen egg lysozyme; HRP, horseradish peroxidase; HSA, heat-stable antigen; MFI, mean fluorescence intensity; PALS, periarteriolar lymphoid sheath; ss, single-stranded; tg, transgene; V, variable.
more interesting possibility to explain these divergent outcomes is that they reflect the different specificities of the tg
used in these studies, which may in turn differ in their regu-
lation (14–17, 20). Because anti-dsDNA Abs from SLE
patients and lupus mice are heterogeneous, and the particu-
lar specificities which are significant in disease are not known,
it will be important to understand these differences (21, 22).

The VH3H9 tg offers an opportunity to study the regu-
lation of a range of anti-dsDNA B cells. Initial studies using
the VH3H9 H chain tg on the BALB/c background dem-
onstrated that neither anti-ssDNA nor anti-dsDNA serum
Abs were elevated over tg(−) BALB/c control sera (23).
When hybridoma panels were generated from the spleens
of VH3H9 tg mice, anti-ssDNA and non-DNA hybridomas
were recovered, but not anti-dsDNA hybridomas (23, 24).
Transfection studies clearly showed that this H chain has
the capacity to generate anti-dsDNA B cells (9). Import-
antly, we have also recovered this specificity in hybridoma
panels generated from VH3H9 M R L-lpr/lpr spleens (10).
The absence of anti-dsDNA hybridomas from BALB/c-derived
panels suggests, therefore, that they are either deleted in the
bone marrow or, if present, cannot be rescued as hybridomas.
To address the mechanisms governing the regulation of
anti-dsDNA B cells in a diverse repertoire and to avoid the
use of L chain tgs altogether, we relied on the fact that
VH3H9 can pair with endogenous V λ1 L chains to gener-
ate anti-dsDNA Abs (9). Thus, we were able to track the
λ1-bearing anti-dsDNA B cells in the context of the di-
verse repertoire of the VH3H9 tg mouse. We have found
that anti-dsDNA B cells are not deleted in the bone mar-
row, but instead exit to populate the spleen. Their unique
surface phenotype suggests that they are both development-
ally arrested and antigen experienced. These cells exhibit
an increased in vivo turnover rate and are localized to the
T–B interface of the splenic white pulp.

Materials and Methods

Mice

BALB/c mice were purchased from Harlan Sprague Dawley
(Indianapolis, IN). VH3H9 tg mice have been described previ-
ously (23). The VH3H9 tg mice have been backcrossed onto
the BALB/c background for at least nine generations, and have been
bred and maintained in the animal facility at The Wistar Institute
(Philadelphia, PA). In all cases, age-matched BALB/c mice or
tg(−) littersmates were used as controls. The presence of the
VH3H9 tg was determined by PCR amplification of tail DNA
with primers specific for VH3H9 (23).

Cell Preparations

Bone marrow, spleen, and lymph node cells were removed
from VH3H9 tg and tgf(−) mice. Single-cell suspensions were
prepared and, where necessary, erythrocytes were removed by
hypotonic lysis.

Flow Cytometry Analysis

Cells (5 × 10^9) were surface stained according to standard
protocols (25). The following Abs were used: RA3-6B2-PE or bi-

Hybridoma Generation

Spleen cells from a VH3H9 tg mouse were stained for B220,
IgM, and CD44, and sorted for IgM^low CD44^high cells (which in-
cludes VH3H9/V λ1 anti-dsDNA B cells) by flow cytometry.
The IgM^low CD44^high cells were then cultured overnight in media
(DMEM/10% FCS) containing CD40 ligand-CD8 fusion pro-
otin (anti-B220), R11-153-FITC (anti-V λ1), R26-46-FITC or
-biotin (anti-V λ total), R8-140-PE (anti-Igκ), 1D3-FITC (anti-
CD19), 7G6-FITC (anti-CD21), Cy34.1-FITC (anti-CD22), 3/23-
FITC (anti-CD40), M1-14-FITC (anti-CD62L, l-selectin),
2G9-PE (anti-I-A^d/I-E^d, M HC class II), M1/69-FITC (anti-
heat-stable antigen [HSA]), IM7-FITC (anti-CD44) (all from
PharMingen, San Diego, CA), LS136-biotin (anti-V λ1), and
JC5.1-PE (anti-Vκ total) (LS136 and JC5.1, gifts from J.
Kearney, University of Alabama, Birmingham, AL; JC5-PE, gift from R.
Hardy, Fox Chase Cancer Center, Philadelphia, PA), polyclonal
anti-IgM-PE and SBA-1-PE (anti-IgD) (Southern Biotechnolo-
gies, Birmingham, AL), B3B4-FITC (anti-CD23) (gift from D.
Conrad, Virginia Commonwealth University, Richmond, Virginia),
and streptavidin-Red670 (GIBCO BRL, Gaithersburg, MD).

All samples were analyzed on a FACScan® flow cytometer
(Becton Dickinson, Mountain View, CA) using Cellquest soft-
ware. 15,000–40,000 events were collected for each sample and
gated for live lymphocytes based on forward and side scatter.

Bromodeoxyuridine Labeling

8-d Labeling. Mice were injected intraperitoneally with 200
μl of 3 mg/ml 5-bromodeoxyuridine (BrdU) (Sigma Chemical Co.,
St. Louis, MO) in PBS every 12 h for 8 d. BrdU staining
was performed essentially as described (26), with the exception
that the cells were not fixed in ethanol. In brief, spleen and bone
marrow cells from mice were isolated and surface stained as
described above. The cells were then fixed and permeabilized with
1% paraformaldehyde containing 0.1% Tween-20. The DNA was
denatured using 10 μM HCl and 100 U/ml DNase I. The incor-
porated BrdU was then detected using an anti-BrdU-FITC Ab
(B44) from Becton Dickinson.

2-h Pulse. Mice were injected intraperitoneally with 200 μl
of 3 mg/ml BrdU in PBS. The spleen and bone marrow cells were
isolated 2 h later and stained as above.

Immunohistochemistry

Spleens were suspended in OCT, frozen in 2-methylbutane
cooled with liquid nitrogen, sectioned, and fixed with acetone.
The spleen sections were stored at −70°C and then stained ac-
cording to the protocol described (27). In brief, the sections were
blocked using PBS/5% BSA/0.1% Tween 20, and then stained
with GK1.5-biotin (anti-CD4), 53-6.7-biotin (anti-CD8), R A 3-
6B2-biotin (anti-B220) (grown as supernatants), and/or anti-Igλ-
alkaline-phosphatase (AP; Southern Biotechnologies). Streptavi-
din–horseradish-peroxidase (HRP; Southern Biotechnologies)
was used as a secondary antibody with the biotinylated reagents
HRP and AP were developed using the substrates 3-amino-9-ethyl-
carbazole and Fast-Blue BB base (Sigma Chemical Co., St.
Louis, MO), respectively.
ELISA assay. The Ig isotype of hybridomas was determined via an indirect solid-phase ELISA assay, using anti-IgH + L (Southern Biotechnologies) as the primary Ab and developing with AP-labeled anti-IgM, -IgG, -Igδ, or -Igλ Abs (Southern Biotechnologies). Binding to dsDNA was detected in a similar manner. In this case, the plates were coated with Avidin-DX (Vector, Burlingame, CA); DNA-biotin was used in place of the primary antibodies. DNA-biotin was prepared as described (9).

Sequence Analysis. The H and L chain variable (V) regions were sequenced from messenger RNA according to the protocol described (29). In brief, cytoplasmic RNA was isolated and constant region–specific primers were used to direct synthesis of cDNA copies of the H (Cμ1) and L (Cα1) chain V regions. The cDNA was then amplified using the constant region primers in conjunction with VH51 or XL primers that hybridize to the 5′-ends of H and L chain V region genes, respectively (29). Amplification products were sequenced by automated analysis (Wistar Institute Nucleic Acid Facility). Sequence translation and comparison was carried out using the Sequencer program and by searching EMBL/GenBank/DDBJ databases.

A ntitumor Ab. The presence of antinuclear antibodies in the supernatants was detected using permeabilized HEP-2 cells as the substrate (Antibodies Incorporated, Davis, CA). Supernatants were then visualized via an indirect solid-phase ELISA assay, using anti-IgH or -Igδ (Southern Biotechnologies) as the primary Ab and developing with AP-labeled anti-IgM, -IgG, -Igδ, or -Igλ Abs (Southern Biotechnologies). The presence of antinuclear antibody (ANA) was taken as evidence for antigen encounter leading to a decrease in Ig density on the B cells. We detect show no evidence of L chain coexpression by flow cytometry; the B cells all express either κ or λ (data not shown). Because low levels of surface Ig are hard to detect using flow cytometry, we took a second approach; B cells were isolated by flow cytometry based on Ig density, cultured in a cocktail mimicking T help (CD40 ligand and rIL-4), and then used to generate hybridoma panels. Anti-dsDNA A hybridomas were recovered that only expressed a λ L chain as detected by ELISA.

Results and Discussion

Anti-dsDNA B cells are present in the periphery of N on autoimmune mice. In this study, we use VH3H9 H chain only tg mice to increase the frequency of anti-dsDNA B cells while maintaining a polyclonal repertoire. Transfection and hybridoma analysis have identified germline Va1 as an L chain that pairs with the VH3H9 H chain to generate an anti-dsDNA Ab (see J558LT and MRL1-45 in Table 1; references 9, 10). Because the VH3H9 H chain tg has been shown to be a good excluder of endogenous H chain rearrangement on the BALB/c background (Table 1; references 23, 30), we can follow the fate of anti-dsDNA B cells in VH3H9 tg mice using anti-λ specific reagents. Several different reagents were used to track λ+ and λ+ B cells (LS136, R 11-153, JC5, and R 26-46). Using these reagents and flow cytometry, we have shown that the majority of λ+ B cells in VH3H9 tg mice are λ1 (79 ± 19%). Therefore, for the remainder of our studies, we have used anti-λ reagents to detect anti-dsDNA B cells. As is shown in Fig. 1, λ+ B cells are present in the bone marrow, spleen, and lymph node. It is also apparent that the levels of Ig on these cells are lower in the periphery compared to λ+ B cells from tg(−) mice (spleen: mean fluorescence intensity [MFI] 52 versus 181; LN: MFI 52 versus 216). Interestingly, Ig levels are also decreased on the λ+ B cells from the bone marrow (MFI 47 versus 197). There is precedent for antigen encounter leading to a decrease in Ig density in other tolerance model systems; for example, anti-hen egg lysozyme (HEL) B cells have a reduced level of Ig when in the presence of HEL (3), but when these B cells are removed from Ag, either by in vivo parking or in vitro cultures, their surface Ig levels increase (31, 32). The decreased Ig density on the anti-dsDNA B cells suggests that these cells are encountering their Ag and that this encounter initially occurred in the bone marrow.

One scenario that could account for the presence of λ+ B cells in the periphery of VH3H9 tg mice is coexpression of a κappa chain or an endogenous H chain to generate a non-dsDNA-binding Ab (10, 15, 33, 34). Indeed, our previous analysis of hybridomas generated from either unmaturated or LPS-activated B cells from VH3H9 BALB/c mice detected a single λ+ hybrid (BALB1-72) that also coexpressed a kappa protein (Table 1). The addition of the second L chain (kappa) disrupted DNA binding, which we suggested most likely spared the B cell from deletion (10). A similar scenario has been described for VH3H9 tg mice bred to Vκ4 tg mice (15). VH3H9/Vκ4 encodes an anti-dsDNA Ig, but no peripheral B cells with this specificity were identified in the tg mice. The fact that all the B cells isolated as hybridomas coexpressed an endogenous L chain was interpreted as evidence for receptor editing (15). In contrast, the λ+ B cells we detect show no evidence of L chain coexpression by flow cytometry; the B cells all express either κ or λ (data not shown). Because low levels of surface Ig are hard to detect using flow cytometry, we took a second approach; B cells were isolated by flow cytometry based on Ig density, cultured in a cocktail mimicking T help (CD40 ligand and rIL-4), and then used to generate hybridoma panels. Anti-dsDNA A hybridomas were recovered that only expressed a λ L chain as detected by ELISA.
Importantly, messenger RNA H + L sequencing analysis showed that these hybridomas exclusively use the VH3H9 tg and Vλ1 (Table 1). The ability to rescue anti-dsDNA B cells with T help-derived factors is intriguing in light of the requirement for T cells in murine SLE (35–37). In addition, this may explain why we, and others, did not previously recover anti-dsDNA B cells from LPS-derived hybridomas (10, 14, 23, 24, 34).

Anti-dsDNA B Cells Are Developmentally Arrested and Show Signs of Antigen Experience. Using flow cytometry, we as-

Figure 1. VH3H9/λ anti-dsDNA B cells are present with a reduced Ig density. Bone marrow (left), spleen (middle), and lymph node (right) cells from Tg(−) (top) and VH3H9 tg (bottom) mice were stained with anti-B220-biotin/streptavidin-Red670 and anti-λ-FITC. MFI is given for the λ+ cells in the boxed region. These are representative plots of n = 19 mice of each genotype.
sessed the developmental and activation status of anti-dsDNA B cells in the spleen. The panel of developmental markers, shown in Fig. 2, includes CD19, CD21/35, CD22, CD23, HSA, and B220. We compared the expression levels of these markers on λ+ B cells from VH3H9 tg mice with those on the tg(−) λ+ B cells, as well as the total B cell population, from tg(−) mice (Fig. 2). B220 (CD45R) increases with maturity and, in conjunction with HSA, has been used to define the immature to mature stages of B cell development (25). HSA is expressed at high levels on immature (newly emerging) B cells and at a lower level on mature B cells in the spleen (26). As is shown in Fig. 2 B, the VH3H9/λ B cells express a slightly reduced level of B220 and a level of HSA intermediate between the HSA high and HSA low cells in the tg(−) spleen. CD22 is expressed at a low level on immature B cells and increases with maturity, whereas CD21/35 and CD23 become surface positive at the mature B cell stage (38–41). The VH3H9/λ B cells have a dramatically reduced level of CD21/35 as well as lower levels of CD22 and CD23 on their surface (Fig. 2 B). Because CD21/35 (complement receptors 1 and 2) and CD22 play a role in modulating the response through the Ig receptor (42–45), the low expression levels of these coreceptors on VH3H9/λ B cells may alter the signaling threshold of these cells when stimulated through membrane Ig. CD19 is a B cell-specific marker that is expressed on all B cells starting at the pro-B cell stage (46). As is shown in Fig. 2 B, CD19 expression on the surface of λ+ B cells in VH3H9 tg mice is higher than on B cells from tg(−) mice. CD40 was also examined and has a similar expression level to tg(−) B cells (data not shown). In contrast to VH3H9 tg mice, the λ+ B cells in tg(−) mice have equivalent levels of all surface markers tested (Fig. 2), suggesting that there is nothing inherently different about B cells with λ L chains. Taken together, these data suggest that the VH3H9/λ B cells are phenotypically immature.

As an indication of activation/antigen encounter, additional cell surface markers, whose differential expression levels have been used to mark a B cell's activation state, were also analyzed. CD44 and MHC class II are molecules whose expression levels increase on activated B cells (47–49). L-selectin expression decreases upon activation, but given that it is also low on immature B cells, it cannot be used to distinguish an immature B cell from a postactivated one (50, 51). The λ+ B cells in VH3H9 tg mice express increased levels of CD44 and MHC class II and decreased...
levels of \( \lambda \)-selectin (Fig. 2 C). Together, these data as well as the increase in cell size (Fig. 2 C), suggest that the anti-dsDNA B cells have encountered antigen.

To determine at what point anti-dsDNA B cells have been arrested in development, flow cytometric analysis of VH3H9/\( \lambda \) bone marrow cells was performed. The majority of \( \lambda \) B cells in the bone marrow of VH3H9 tg mice express similar levels of the markers B220, HSA, CD21, CD22, CD23, CD44, and \( \lambda \)-selectin as the tg(–) bone marrow B cells (compare the bold lines in Fig. 2 D). Additionally, in comparison to the bone marrow, the VH3H9/\( \lambda \) B cells in the spleen have altered their expression level of B220, HSA, CD22, CD23, and \( \lambda \)-selectin, consistent with their continued maturation (compare the thin line to the bold line in Fig. 2 D). These data suggest that B cell development proceeds in the bone marrow for the VH3H9/\( \lambda \) cells in a similar fashion to \( \lambda \) cells from tg(–) mice, and furthermore, that the \( \lambda \) anti-dsDNA B cells in the spleen have matured more than the majority of bone marrow B cells.

VH3H9 tg mice contain, in addition to anti-dsDNA B cells, a population of non-DNA B cells in the repertoire that allow us to control for and distinguish effects which are due to autoreactive specificity versus those that are due to the presence of the transgene (10, 23). The presence of non-DNA B cells in VH3H9 tg mice would predict that not all of the B cells in these mice will exhibit the immature/activated phenotype. Indeed, comparing the surface phenotype of the \( \kappa \) cells in the VH3H9 tg mice with B cells from tg(–) mice shows that the majority of the VH3H9 \( \kappa \) B cells express cell surface densities equivalent to their tg(–) counterparts for both the developmental markers (CD21/35, CD22, CD23, B220, and HSA; Fig. 2 B) as well as activation markers (CD44, \( \lambda \)-selectin, and cell size; Fig. 2 C). In contrast, CD19 and MHC class II are slightly elevated on all of the VH3H9 cells (Fig. 2, B and C), implying that their alteration may be due to the presence of VH3H9 tg per se, and not to their autoreactive specificity. Furthermore, as transfection studies and L chain repertoire analysis of hybridoma panels from VH3H9 tg MRL-1pr/lpr mice have revealed, there are \( \kappa \) L chains in addition to \( \lambda \), which can pair with the VH3H9 H chain to generate anti-dsDNA B cells (8–10). We predicted that these B cells would also be regulated and exhibit an altered surface phenotype. Consistent with this, there is a small population of VH3H9 \( \kappa \) B cells that exhibit a phenotype similar to the VH3H9/\( \lambda \) B cells: CD21/35\(^{low} \), CD22\(^{low} \), CD23\(^{low} \), CD44\(^{high} \), and \( \lambda \)-selectin\(^{low} \) (note the shoulders in Fig. 2 B and C).

In summary, the VH3H9/\( \lambda \) anti-dsDNA B cells have a unique cell surface phenotype, as does a subpopulation of VH3H9/\( \kappa \) B cells. They show evidence of activation (CD44\(^{high} \), \( \lambda \)-selectin\(^{low} \), and increased cell size), suggesting that they have been exposed to Ag. In addition, they appear developmentally arrested in that they express reduced levels of CD21/35, CD22, CD23, \( \lambda \)-selectin, and B220, and intermediate levels of HSA. These data suggest that anti-dsDNA B cells have encountered their Ag while still at an immature stage, resulting in arrested development with respect to some markers and a concurrent change in the expression of activation markers. In support of this, there is a reduced level of surface Ig in the bone marrow (Fig. 1). A similar immature phenotype (low levels of CD21/35, CD22, CD23, and \( \lambda \)-selectin) was seen in the anti-HEL/membraneHEL mice in the context of a bcl-2 tg, whereas in the absence of the bcl-2 tg, the anti-HEL B cells were deleted.
Anti-dsDNA B Cells Have a Reduced Lifespan. The in vivo lifespan of a B cell can be estimated by continuously labeling mice with the thymidine analogue BrdU, and then measuring the incorporation of BrdU-labeled cells into the splenic population (4, 52). A population that is rapidly turning over will be replaced more quickly with labeled cells from the bone marrow. Studies of BALB/c splenic B cell turnover rates have estimated that B cells have an average lifespan of 3–4 wk (4). To estimate the lifespan of anti-dsDNA B cells, the frequency of BrdU-labeled cells is significantly decreased in VH3H9 Ig low B cells (62%). It is possible that the frequency of autoreactive B cells in tg(52) is too low to detect by these means, underscoring the advantage of using reagents to detect these B cells (as we do for VH3H9 tg mice). The Ig low B cells are present, although at a decreased in vivo lifespan (4, 13). Here we have shown that when anti-dsDNA B cells are present in a polyclonal repertoire, they are short lived. Importantly, in another anti-dsDNA Ig l1 tg model in the bone marrow (2, 32). Thus, the bcl-2 tg protects B cells from deletion, but does not protect them from maturational arrest. The anti-dsDNA B cells are unique in that they are present in the periphery with an immature phenotype in the absence of a bcl-2 tg.

To assess the lifespan of the anti-dsDNA population of cells, we examined BrdU incorporation in the Ig low subset. Fig. 3 A demonstrates that among the Ig low cells, the frequency of BrdU-labeled cells is significantly higher in VH3H9 Ig low B cells than in Ig high B cells (63.1 ± 5.6% versus 9.5 ± 0.4%), suggesting that the anti-dsDNA B cells have a decreased in vivo lifespan. Alternatively, the increased BrdU uptake in this population could be due to the active proliferation of the Ig low B cells in vivo. To address this, we pulsed VH3H9 Ig low B cells with BrdU for 2 h and then measured the uptake of BrdU in the splenic B cell populations. This is a time point when actively proliferating cells will take up BrdU, but is not a long enough period for replacement of cells from the bone marrow pool (52). We were unable to detect BrdU label in the Ig low B cells in VH3H9 Ig low B cells spleens. Labeling was analyzed and detected in the rapidly dividing populations of the bone marrow, thus ensuring that the mice did receive BrdU (data not shown). Together, these data suggest that the increased BrdU labeling of the VH3H9 Ig low B cells is due to their decreased in vivo lifespan.

Given that there are many k L chains in addition to l1 that can pair with the VH3H9 H chain to generate anti-dsDNA B cells, we predicted that they too may exhibit a decreased lifespan. Since we do not have L chain-specific reagents to detect these B cells (as we do for l1), we used the phenotype of Ig l1 to distinguish these cells. The Ig low cells are included within the Ig low cells, comprising 20–30% of this subset. Ig l1 cells in Ig low and VH3H9 Ig low B cells were defined by gating on cells stained with either IgM and IgD (Fig. 3 B) or with only IgM (data not shown); both approaches yielded similar results. As shown in Fig. 3 B, the majority of the B cells in VH3H9 Ig low B cells have approximately the same amount of BrdU incorporation as the Ig low B cells. However, when we gate on the Ig low subset of cells, 57.7 ± 5.1% of the Ig low B cells are labeled. These data are consistent with the idea that there are Ig l1 k1 cells in VH3H9 Ig low B cells that are dsDNA reactive, and these also have a rapid turnover rate. Interestingly, when we look in Ig low mice, the Ig l1 B cells are present, although at a much reduced frequency (<5% of the B cells). The Ig l1 cells that we do detect have a slight increase in BrdU labeling (16%); however, this is much lower than that seen in the VH3H9 Ig l1 B cells (62%). It is possible that the frequency of autoreactive B cells in Ig low mice is too low to detect by these means, underscoring the advantage of using the VH3H9 Ig low B cells to track these cells.

In the HEL B cell tolerance model, an increased in vivo turnover rate has been hypothesized to be a mechanism that maintains tolerance to self Ags (4). However, it is unclear whether this is dependent upon competition with nonautoreactive B cells or an intrinsic property of anergic B cells (4, 13). Here we have shown that when anti-dsDNA B cells are present in a polyclonal repertoire, they are short lived. Importantly, in another anti-dsDNA Ig l1 tg model...
Studies are underway to define the role that Fas-mediated apoptosis plays in the elimination of anti-dsDNA B cells once they have been reactivated and to determine if they form antibody-forming cells at this site in lpr/lpr mice.

Increased Frequency of λ + B Cells in V H 3 H 9 tg Mice. The λ + B cells are not only present in the periphery of V H 3 H 9 tg mice, but they are present at a twofold higher frequency than in the tg(−) controls, despite the fact that V H 3 H 9/λ1 B cells are autoreactive and have had a decreased turnover rate (Fig. 5). What could account for this seemingly surprising result? Pulsing mice with BrdU showed that the increased number of V H 3 H 9/λ B cells is not simply due to their proliferation in the periphery (data not shown). Another possibility is that the V H 3 H 9/λ B cells are positively selected (on some unidentified ligand) in the bone marrow. In support of this, there is a twofold increase in λ + B cells in the V H 3 H 9 tg bone marrow over tg(−) bone marrow (Fig. 5). Alternatively, the increased λ frequency may be the consequence of receptor editing where λs represent the end result of multiple L chain rearrangement attempts (19, 33). The scenario we favor for an autoreactive Ig in V H 3 H 9 tg mice is that after rearrangement at the k loci is exhausted, λ rearrangement occurs, completing receptor editing. The λ + B cells that are generated are not deleted; rather, they persist in a compromised state.

Interestingly, when we compare the frequency of λ-expressing B cells in V H 3 H 9 and tg(−) mice, the frequency is highest in the bone marrow and then decreases in the spleen. This is indicative of the loss of B cells from the bone marrow and recruitment into the long-lived splenic B cell pool (60). The frequency of λs changes only slightly between the spleen and lymph node in tg(−) mice; however, it decreases by half from the V H 3 H 9 spleen to the lymph node.
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