Autoreactive B Cells in the Marginal Zone that Express Dual Receptors

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Abstract

Allotype and isotype exclusion is a property of most lymphocytes. The reason for this property is not known but it guarantees a high concentration of a single receptor, and threshold numbers of receptors may be required for efficient positive and negative selection. Receptor editing compromises exclusion by sustaining recombination even after a functional receptor is formed. Consequently, B cells expressing multiple receptors arise. We have studied such B cells in which one of the two receptors is anti-self, and find that these partially autoreactive B cells accumulate in the marginal zone. The restriction of these cells in this location may help to prevent them from undergoing diversification and developing into fully autoreactive B cells.

Key words: editing • autoimmunity • antinuclear antibody • tolerance • allele exclusion

Introduction

Exclusion of a L chain or H chain allele and expression of just one L chain isotype (κ or λ) results from a signal(s) that terminates recombination when V gene rearrangement produces a functional receptor (1). Thereby, rearrangement of other alleles or isotypes is prevented as are rearrangements that delete functional V genes. Inclusion, resulting from concomitant productive rearrangements, is kept to a minimum by the extremely high frequency of nonproductive or aberrant rearrangements (2). Isotype inclusion is thought to be further reduced by the slow rate of λ rearrangement relative to κ (3, 4).

Receptor editing reinitiates or sustains recombination even though a functional, albeit autoreactive, receptor is expressed (5). Editing is an efficient mechanism of achieving self-tolerance because it deletes or inactivates autoreactive V genes (6). However, editing compromises exclusion when productive rearrangements occur at alleles or isotypes other than those that code for the autoantibody. Yet B cells with multiple receptors (7) or that secrete more than one Ab (8) are rare. There are multiple ways of eliminating multireactive B cells. First, multireactive B cells that result from overzealous editing will be partially autoreactive and may, therefore, continue to edit. Additional editing can now delete or inactivate the V gene(s) that contributes to autoreactivity, rendering this B cell either self-tolerant or defunct. Second, not all of the productive rearrangements may be expressed. We first described this case (referred to as "phenotypic exclusion") in anti-DNA transgenic mice. Self-tolerant B cells were observed that express two κ chains but only one, the editor, was expressed on the cell surface or secreted by hybridomas derived from these mice (9). In this case autoreactivity was minimized by H chain/L chain dimerization that favors the L chain editor. In the current study we describe an instance in which multireactive B cells do arise from editing. These partially autoreactive B cells that express both κ and λ are directed to the marginal zone (MZ).*

B cells that coexpress κ and λ are found among the hybridomas derived from LPS-activated spleen cells from an anti-DNA transgenic mouse, 3H9H/56R (10). Of the two antibodies secreted by these hybridomas, only the transgenic H chain/λ combination binds DNA; the other Ab is comprised of the transgenic H chain and a κ L chain that can veto DNA binding, and we call such a L chain "an editor." These young anti-DNA transgenic mice do not secrete anti-DNA Ab, suggesting that these partially autoreactive B cells are regulated from converting to Ab-secreting cells. Here we have studied the frequency, phenotype, and location of κ/λ anti-DNA B cells.

Materials and Methods

Mice. Generation of three different heavy chain site-directed transgenic (sd-tg) mice, 3H9, 3H9H/56R, and 3H9H/56R/76R, is described (10). 3H9H/56R mice were also backcrossed for 8–10 generations to CB17. BALB/c and CB17 mice

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**Abbreviations used in this paper:* MZ, marginal zone; sd-tg, site-directed transgenic.

were purchased from The Jackson Laboratory. Unless specified, all the mice used in the experiments were between the ages of two to four months.

Flow Cytometry Analysis. Single-cell suspensions were prepared from spleen or bone marrow as described previously (11). Antibodies used for FACS® were: FITC-conjugated goat anti–mouse κ (Fisher Scientific), biotin-conjugated anti-λ (R26–46), FITC-conjugated anti-CD21/CD35 (7G6), PE-conjugated anti-CD23 (B3B4), FITC-conjugated anti-CD43 (S7), allophycocyanin (APC)-conjugated anti-B220 (RA3–6B2; BD PharMingen), PE-conjugated anti-κ (187.1), FITC-conjugated anti-IgD (11–44–2), FITC or TXRD-conjugated goat anti-IgM (Southern Biotechnology Associates, Inc.), and Tri-color-conjugated anti-CD19 (6D5; Caltag). Anti-idiotype Ab 1.209 has been described previously (9). It was conjugated to biotin or Alexa 488 using FluoReporter Mini-Biotin-XX Protein Labeling Kit and Alexa Fluor™ 488 Protein Labeling Kit (Molecular Probe, Inc.). Alexa 488–conjugated anti-IgM^a and Biotin-conjugated anti-IgM^b were gifts from Dr. John Kearney (University of Alabama at Birmingham, Birmingham, AL). Biotin-conjugated antibodies were revealed by PE-conjugated Streptavidin (Molecular Probe, Inc.) or Cy-chrome-conjugated Streptavidin (BD PharMingen). To avoid cross-talk between the antibodies (anti-κ can bind to anti-λ, but anti-λ does not bind to anti-κ), the κ/λ double staining was done by staining the cells with anti-κ first. After washing with FACS® buffer (PBS/1%BSA/0.05% sodium azide), the cells were stained with biotin-conjugated anti-λ (BD PharMingen) followed by PE or Cy-chrome–conjugated Streptavidin. Stained cells were analyzed using FACScan™ or FACSVantage™ flow cytometer (Becton Dickinson) and CELLQuest™ (Becton Dickinson) software.

Immunohistochemistry. Spleens were suspended in OCT compound (Sakuro Finetek USA, Inc.) and frozen in liquid nitrogen, sectioned in 8-μm sections and stored at −20°C until use. The sections were blocked with PBS/5% BSA/0.1% Tween 20 and stained with alkaline phosphatase (AP)-labeled goat anti-mouse IgM (Southern Biotechnology Associates, Inc.) and biotin-conjugated MOMA-1 (Bachem Bioscience). After washing with PBS/0.1% Tween 20, the sections were incubated with horseradish peroxidase (HRP)-conjugated Streptavidin (Jackson ImmunoResearch Laboratories). AP and HRP were developed with substrates Fast BB Blue base and 3-amino-9-ethylcarbazole (Sigma-Aldrich), respectively.

Results

Properties of B Cell Populations in Mice with Heavy Chain Transgenes of Different DNA Binding Affinity. Previous studies using transgenic anti-DNA mice have shown that autoreactive B cells are actively regulated by receptor editing, a process that involves replacement of L chain as well as H chain to veto DNA binding (6, 12). To study the effect of the affinity of autoreactive B cells receptor on editing, we made mice with three different site-directed H chain transgenes (3H9H, 3H9H/56R, 3H9H/56R/76R sd-tgs) encoding antibodies that bind to DNA with different affinities (10). All three mice carry the H chain as a sd-tg, while the L chains are derived from endogenous L chain rearrangements. The number of peripheral B cells decreases as the affinity for DNA of the H chain tg increases. The IgM/IgD double-positive population in spleen of the sd-tg series ranges from nearly normal levels for 3H9H to ~75% of normal values for the higher affinity sd-tgs. The difference is much greater in bone marrow where the B cell levels of the 3H9H/56R and 3H9H/56R/76R sd-tgs are just 20% of normal mouse bone marrow (Fig. 1 A). The extent of H chain editing in the sd-tg series was assessed by staining B cells with a mAb specific for the VH3H9. This reagent, 1.209, binds 3H9, 3H9/56R, and 3H9/56R76R H chains in association with many different L chains (9). The 1.209 reagent stains a substantial number of B cells in 3H9H sd-tg mice (Fig. 1 B). The absolute frequency of 1.209-expressing B cells is less than total IgM/IgD B cells in 3H9H/56R and 3H9H/56R/76R sd-tg mice. The frequency of loss of the H chain transgene in the hybridomas made from these mice is also correlated with affinity for DNA (10). Together, these results indicate extensive loss of sd-tg expression in the higher affinity anti-DNA sd-tg mice.

L Chain Isotypes in H Chain Transgenics. κ and λ expression in peripheral B cells showed striking differences between the sd-tgs (Fig. 2 A). 3H9H mice have κ and λ single-positive B cell populations similar to BALB/c mice. The only discernable difference is that the λ expression level is lower in 3H9H mice. We attribute this to the DNA binding capacity of the 3H9H/λ1 combination (13). The affinity for DNA of this combination is relatively high and we assume that B cells with this combination are edited or downregulated.

3H9H/56R mice with an anti-DNA H chain of higher affinity have 25% fewer κ and even fewer λ single-positive B cells than 3H9H (Fig. 2 A). This sd-tg is unique in that it has a large population of B cells that express both κ and λ. The κ/λ double-positive B cells constitute 16~34% (average 23.8 ± 8.2%, $n = 11$) of the B220$^+$ spleen B cell population in 3H9H/56R mice between 2 and 5 mo of age, as compared with only 2.1 ± 1.0% ($n = 9$) of such cells in the B220$^+$ population from nontransgenic littermates (Fig. 2 B). This type of B cell is not found in the highest affinity sd-tg, 3H9H/56R/76R (Fig. 2 A). However, the κ/λ phenotype is seen mainly in B cells that express the 3H9H/56R sd-tg (see below). The corresponding sd-tg population in 3H9H/56R/76R is extremely low due to extensive VH replacement (Fig. 1 B) and this might explain the low frequency of κ/λ B cells.

The level of λ on the κ/λ B cells is lower than the λ-only B cells in the control mice (Fig. 2 A). This could mean that receptors are downregulated in these B cells or that a mechanism limits the total L chain that can be expressed on the surface of a B cell. We favor the latter alternative because surface IgM levels are higher than most of the analogous κ-only B cell populations (Fig. 2 C). It is likely that surface expression levels are limited by the H chain levels, in which case it might be predicted that excess L chain would be secreted. This was found to be the case in the original κ/λ plasmacytoma (14).

Increase of the MZ B Cell Population in H Chain Transgenic Mice. The marginal zone (MZ) has been shown to be enriched in self-reactive B cells in certain transgenic models

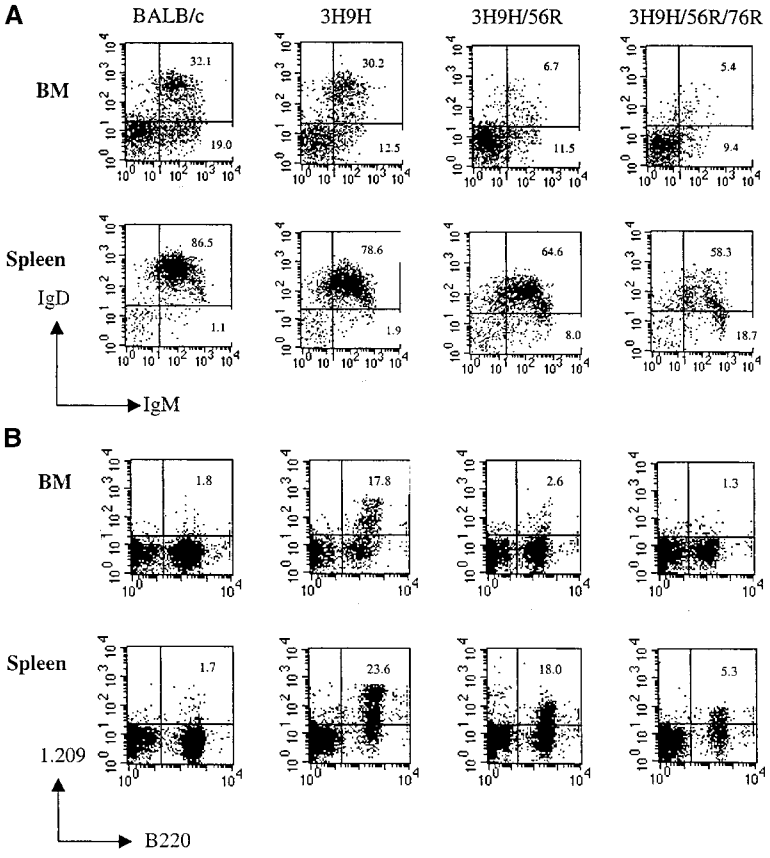

Figure 1. FACS® analysis of mature B cells in anti-DNA H chain sd-tg mice. (A) Splenic and bone marrow cells were isolated from 3H9H, 3H9H/56R, and 3H9H/56R/76R sd-tg mice. Cells were stained with antibodies against B220, CD43, IgM, and IgD. All analyses were performed on the lymphocyte-gated population. IgM^+IgD^+ cells were plotted from the $B220^+CD43^-$-gated population. (B) Recognition of transgenic H chain–bearing cells by the anti-idiotypic Ab, 1.209, which recognizes the 3H9 H chain in combination with most L-chain. Cells were double-stained with anti-B220 and 1.209. Data were plotted and percentages calculated from the lymphocyte-gated population. These results are representative of four independent experiments.

and autoimmune mice (15). We investigated whether mice expressing anti-DNA Ig transgenes have similar features. Fig. 3 A shows increases of MZ B cells ($CD21^{high}$ and $CD23^{low}$) in all three transgenic mice. Immunohistochemical staining confirms the results of FACS® analysis (Fig. 3 B).

Of the three sd-tg lines, 3H9H/56R mice have the largest population of MZ B cells. As 3H9H/56R mice are also enriched in the κ/λ population we wondered whether there was a correlation between this population and the MZ population. This is the case, the κ/λ double-positive B cells comprise the majority of the MZ B cells, defined as $CD21^{high}$, $CD23^{low}$, and IgD^{low}. There is also a small population of MZ B cells in 3H9H/56R that express only κ chains (Fig. 4). In comparison, the MZs of 3H9H and 3H9H/56R/76R mice are made up of B cells expressing one type of L chain (Fig. 2 A).

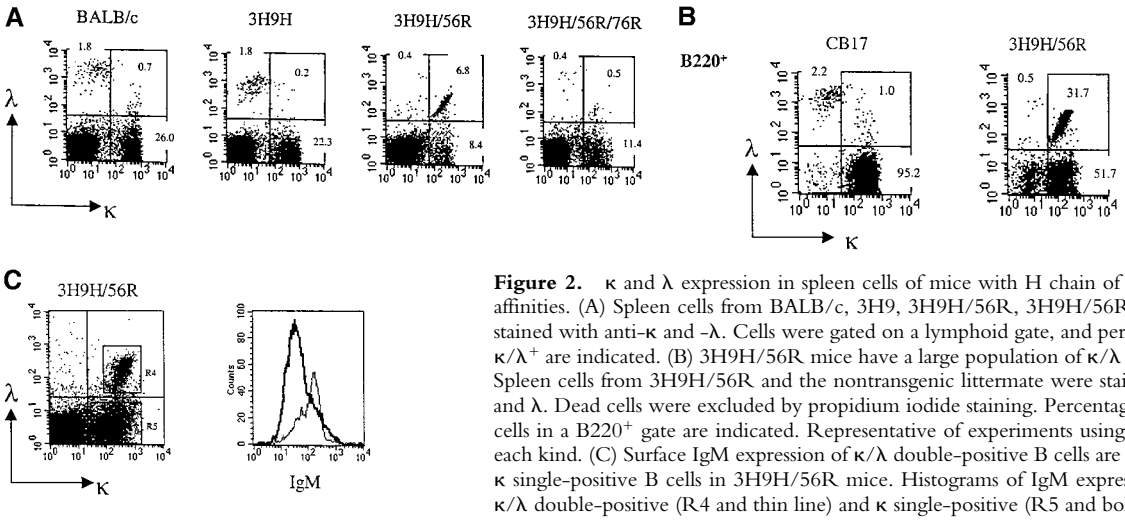

Figure 2. κ and λ expression in spleen cells of mice with H chain of different DNA binding affinities. (A) Spleen cells from BALB/c, 3H9, 3H9H/56R, 3H9H/56R/76R sd-tg mice were stained with anti-κ and -λ. Cells were gated on a lymphoid gate, and percentages of κ^+, λ^+, and κ/λ^+ are indicated. (B) 3H9H/56R mice have a large population of κ/λ double-positive B cells. Spleen cells from 3H9H/56R and the nontransgenic littermate were stained with anti-B220, κ, and λ. Dead cells were excluded by propidium iodide staining. Percentages of κ^+, λ^+, and κ/λ^+ cells in a $B220^+$ gate are indicated. Representative of experiments using three different mice of each kind. (C) Surface IgM expression of κ/λ double-positive B cells are higher than most of the κ single-positive B cells in 3H9H/56R mice. Histograms of IgM expression are shown for the κ/λ double-positive (R4 and thin line) and κ single-positive (R5 and bold line) population.

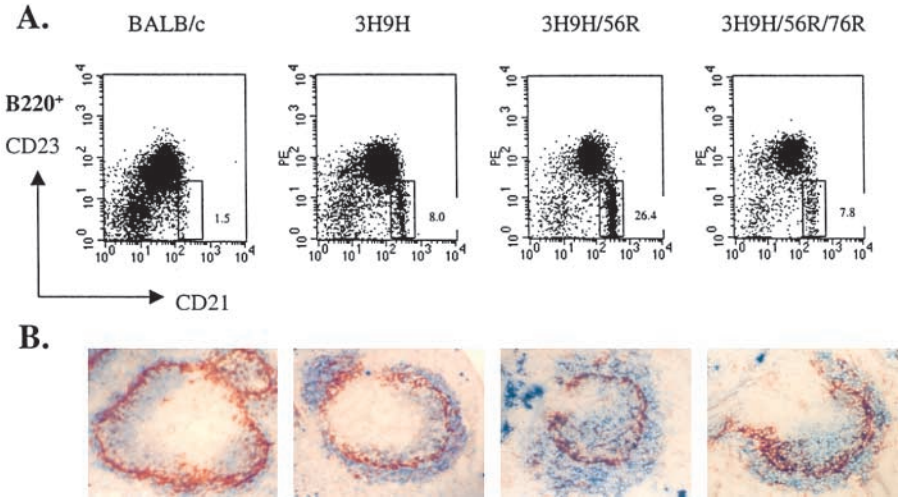

Figure 3. Increase of MZ B cells in the H chain transgenic mice. (A) Spleen cells from BALB/c, 3H9, 3H9H/56R, 3H9H/56R/76R sd-tg mice were stained with anti-B220, -CD21, and -CD23. Percentages of $CD21^{high}$ and $CD23^{low}$ populations in the $B220^+$ gate are indicated. (B) Increase of MZ B cells in the H chain sd-tg mice shown by immunohistochemistry. Spleen sections of each mouse were stained with anti-IgM that stains both MZ and follicular B cell (blue) and MOMA-1 that detects metallophilic marginal macrophage (red).

H Chain Expression in 3H9H/56R Mice. The 3H9H/56R mouse has B cells that express the sd-tg and B cells that have edited the sd-tg. The latter population expresses a diverse H chain repertoire derived from endogenous genes. We wished to determine which population was associated with the κ/λ phenotype.

We first studied the expression of the H chain transgene according to allotype. The 3H9H/56R sd-tg (IgM^a) was crossed to CB17 mice (IgM^b). Staining of CB17 3H9H/56R transgenic spleen cells with anti-IgM^a and anti-IgM^b reveals that >90% of the B cells express the transgene allotype. The rest express IgM^b (2.2%) and a few express both allotypes (4.6%; Fig. 5).

Because IgM^a B cells may not express the sd-tg VH gene due to VH replacement (12), we determined how many B cells have edited the 3H9/56R H chain. Just 23% of B cells in 3H9H/56R mice are Id positive (Fig. 6 A), indicating extensive VH replacement of the 3H9H/56R sd-tg. However, the 1.209 mAb does not bind VH3H9 in association with all L chains. In addition, B cells that have a low density of receptors may not be revealed due to the sensitivity of the anti-Id Ab. Thus, we cannot assess the exact extent of VH editing.

However, the majority (83%) of the Id-positive B cells in 3H9H/56R mice are $CD21^{high}$, and only 40% of the $CD21^{high}$ B cells are Id negative. Thus the unedited B cells have the MZ phenotype. The κ/λ double-positive B cells are all Id-positive (Fig. 6 B).

Accumulation of κ/λ Double-positive Cells in the Periphery. The high frequency of κ/λ double-positive B cells is not seen in the bone marrow. There are major losses in all of the IgM and IgD positive subsets ($IgM^{low}B220^{low}$, immature $IgM^{high}IgD^{low}B220^{low}$, and mature/recirculating $IgM^+IgD^{high}B220^{high}$) in the 3H9H/56R mice (Fig. 7, A and B). Neither the immature nor the mature/recirculating B cell populations in the bone marrow has a significant percentage of κ/λ double-positive B cells (Fig. 7 C). There are also no significant numbers of Id-positive $B220^+$ bone marrow B cells in the transgenic mice (Fig. 7 D). This may reflect negative selection of those self-reactive B cells with the H chain transgene. However, with age there is a gradual increase of κ/λ double-positive and Id-positive population in the MZ of the spleen (Fig. 8). Such an increase could be due either to their expansion in the periphery or to longer life span of these cells.

Discussion

Hybridoma panels from 3H9H/56R sd-tg mice reveal B cells with an unusual phenotype. Some 3H9H/56R B cells produce an anti-DNA Ab along with an Ab that does not bind DNA. The former is a combination of 3H9/56R and λ, the latter is a combination of 3H9/56R and a κ editor such as Vκ38c (10). These B cells are paradoxical in that they have been subjected to the major self-tolerance mechanism, receptor editing, yet they express an anti-self Ab.

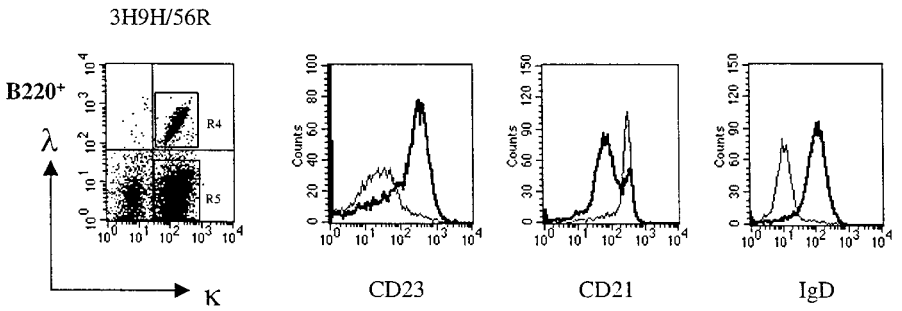

Figure 4. The κ/λ double-positive B cells in 3H9H/56R are MZ B cells. Histograms of CD23, CD21, and IgD expression are shown for the κ/λ double-positive (R4 and thin line) and κ single-positive (R5 and bold line) population. Representative of experiments using three different mice.

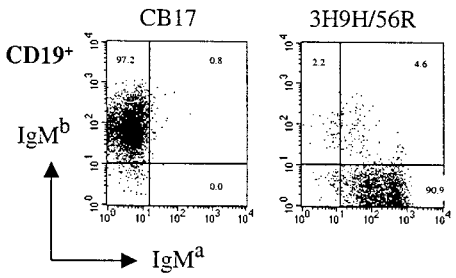

Figure 5. Allelic exclusion by the 3H9H/56R H chain transgene. Spleen cells from 3H9H/56R mice and the nontransgenic littermate were stained with anti-CD19, -IgM^a, and -IgM^b. Percentages of IgM^{a+} (the transgenic allele) and IgM^{b+} (the endogenous allele) cells in the CD19$^+$ gate are indicated. Representative of two independent experiments using a different mouse of each kind.

Here we show that the frequency of κ/λ B cells is high among MZ B cells of this sd-tg and the κ/λ coexpressers also express the 3H9/56R H chain (Fig. 6 B). In addition, Vκ-specific PCR analysis of κ/λ B cells from 3H9H/56R sd-tg mice show that they are enriched in Vκ editors such as Vκ21D (unpublished data). Thus these unique hybridomas have a counterpart in the MZ B cells of this sd-tg.

We propose that these κ/λ B cells arise through the following pathway: the B cell repertoire of 3H9H/56R sd-tg mice, as it arises in the bone marrow, is largely anti-self because most 3H9/56R/Vκ combinations bind DNA. Hence most of the sd-tg B cells will be edited, and because Vκ editors are rare, the cells usually undergo several editing attempts before they succeed in achieving self-tolerance. During the course of this search process, λ rearrangement begins (4) or reaches a significant rate (3) and 3H9/56R/λ B cells are produced. This combination also binds DNA (6), consequently further editing is required. Because the κ/λ B cells always include a κ editor, this B cell seems to have achieved self-tolerance. The alternative pathway, first the κ editor, then λ, is less likely. We do observe B cells

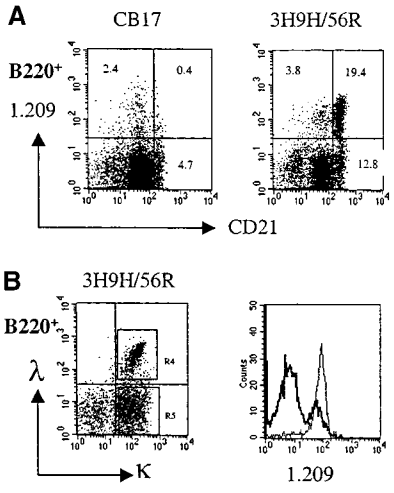

Figure 6. Detection of Id-positive B cells in 3H9H/56R mice. (A) The majority of Id-positive B cells in 3H9H/56R mice are in the MZ. Spleen cells from 3H9H/56R mice and the nontransgenic littermate were stained with anti-B220, -CD21, and 1.209 anti-Id antibodies. Percentages of CD21^{high} and Id-positive cells in the B220$^+$ gate are indicated. Representative of experiments using three different mice of each kind. (B) The κ/λ double-positive B cells in 3H9H/56R are Id positive. Histogram of 1.209 anti-Id staining are shown for the κ/λ double-positive (R4 and thin line) and κ single-positive (R5 and bold line) populations. Representative of experiments using three different mice.

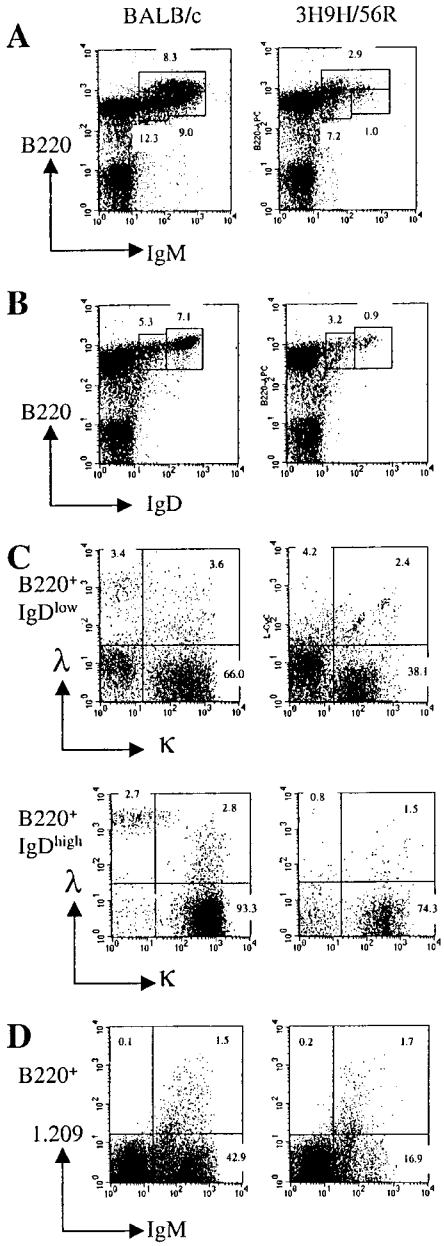

Figure 7. Loss of immature and mature/recirculating B cells and absence of κ/λ and Id-positive B cells in the bone marrow of 3H9H/56R mice. (A and B) Bone marrow cells from 3H9H/56R mice and the nontransgenic littermate were stained with anti-B220, -IgM, and -IgD. Percentages of B cells in the subsets of IgM^{low}B220^{low}, IgM^{high}B220^{low}, IgM$^+$B220^{high}, IgD^{low}B220^{low}, and IgD^{high}B220^{high} are indicated. (C) Bone marrow cells gated from IgD^{low}B220^{low} and IgD^{high}B220^{high} are shown for their light chain expression. Percentages of κ$^+$, λ$^+$, and κ/λ$^+$ cells are indicated. (D) Bone marrow cells are stained with anti-B220, -IgM, and 1.209. Percentages of Id$^+$, IgM$^+$, and Id/IgM$^+$ cells in a B220$^+$ gate are indicated.

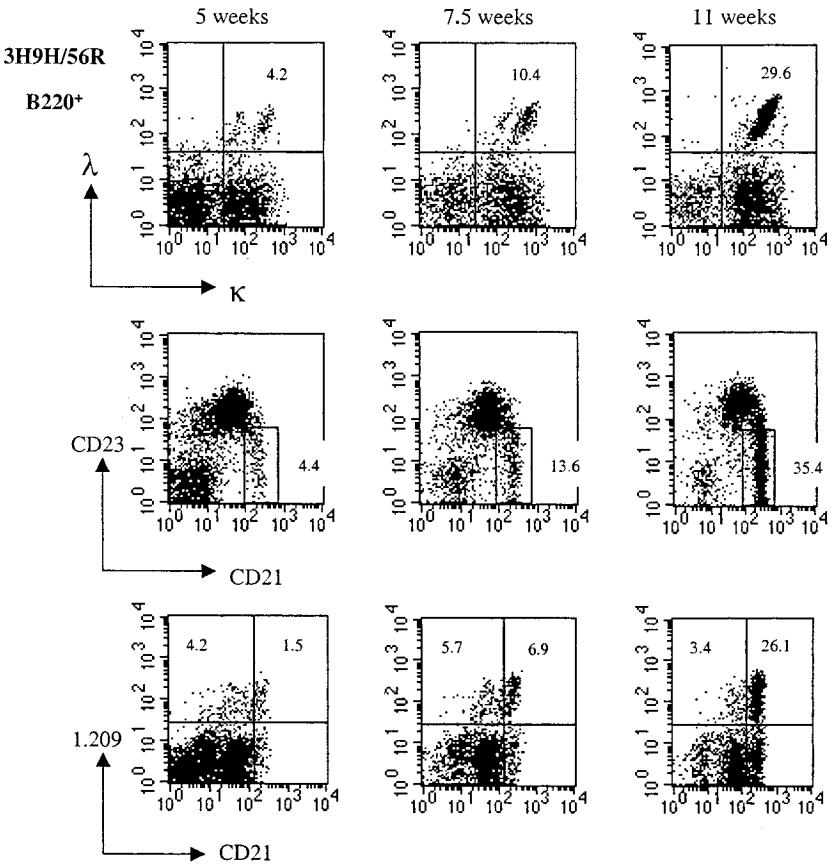

Figure 8. Increase of κ/λ double-positive, MZ, and Id-positive B cells in the spleen of 3H9H/56R mice with age. Spleen cells from 3H9H/56R mice of age 5, 7.5, and 11 wk were stained with anti-B220, -κ, -λ, -CD21, -CD23, and 1.209 anti-Id antibodies. Percentages of κ/λ double-positive B cell, $CD21^{high}$ and $CD23^{low}$ MZ B cells, and Id-positive cells in the $B220^{+}$ gate are indicated.

that express 3H9/56R with just a κ editor and these B cells are efficiently edited, and the 3H9/56R/Vκ-editor Ab does not bind DNA (10). We would expect that this VH/VL receptor would terminate further rearrangement and, thereby, prevent formation of a κ/λ B cell.

The κ/λ phenotype is unique to 3H9H/56R; neither the lower (3H9H) nor the higher (3H9H/56R/76R) affinity VH sd-tg mice have significant numbers of such B cells. We explain the difference in two ways: the 3H9 VH is more easily edited than 3H9/56R. Between 30 and 40% of the Vκ repertoire either vetoes or modifies DNA binding compared with only 4% in the case of 3H9/56R (10). Hence, fewer editing attempts of 3H9H B cells are required on average, and it is less likely that a productive λ will arise before a successful κ editor. The low frequency of κ/λ in 3H9H/56R/76R requires a different explanation, one that must depend on the inability of any Vκ to completely veto DNA binding by this high affinity VH. Even the efficient editors of 3H9/56R in combination with the 3H9/56R/76R H chain bind DNA moderately well (10). A 3H9/56R/76R/λ B cell will now be fully autoreactive regardless of which κ is coexpressed. Such a B cell will continue editing and will eventually lose functional V genes. Why then are B cells with Vκ editors observed among the hybridomas or the B cells of the 3H9H/56R/76R mice (10)? One possibility is that the low affinity for DNA of a 3H9/56R/76R Vκ editor receptor directs a B cell to anergy. Another possibility is that these B cells are no longer subject to regulation. Indeed these sd-tg mice, unlike either 3H9H or 3H9H/56R sd-tg mice, secrete high levels of anti-DNA as early as 3 wk of age (unpublished data).

The difference between 3H9H/56R and 3H9H/56R/76R with respect to the κ/λ phenotype implies that the multireactive B cell is immune from further rearrangement, i.e., it has reached a state of tolerance as far as the editing mechanism is concerned. Moreover, young BALB/c sd-tg mice do not have significant levels of anti-DNA (although older mice express higher levels of anti-DNA in their sera; unpublished data). We considered that 3H9H/56R κ/λ cells might be regulated in an alternative fashion such as by inactivation (16) or sequestration from self-antigen (17). However, as shown in Figs. 2 C and 4, 3H9H/56R κ/λ cells do not have the reported phenotype of anergic B cells such as IgM^{low} (16, 18), nor do they have the markers of B1 cells such as CD5 (data not shown). B1 cells are thought to be directed to sites such as the peritoneal cavity by virtue of their self-reactive receptors. In this environment these B cells are not exposed to the relevant self-antigen and autoimmunity is prevented by lack of activation (17). But this is not the destination of 3H9H/56R κ/λ cells. Instead, they are routed to the MZ. MZ B cells have been shown to be hyper-reactive to antigens and mitogens. This population is enriched in B cells with certain specificities such as

for phosphorylcholine. These properties have suggested that MZ B cells represent the "first-line of defense" against pathogens. After foreign antigen exposure, MZ B cells can quickly migrate from their original site into places such as T cell zone and B cell follicles (for a review, see reference 19). However, constant exposure to self-antigens seems unable to trigger such a response. This may due to low affinity or lack of a costimulatory signal. In case of 3H9H/56R κ/λ cells, the low affinity is generated by the presence of a κ editor. Another important feature of MZ B cells is their T cell–independent response (20). Because somatic mutation, class switch, and clonal expansion yield disease-associated autoantibodies (21, 22), it is clearly undesirable for an autoreactive B cell to receive T cell help that leads to a secondary immune response.

The 3H9H/56R κ/λ B cell may represent a form of tolerance different from that achieved by phenotypic exclusion of an autoreactive receptor (9). Phenotypic exclusion was originally discovered in the 3H9H/Vκ4 mouse in which many B cells express both the transgene-encoded Vκ4, which with 3H9 H chain forms an anti-DNA, and an endogenous κ chain. Hybridomas from 3H9H/Vκ4 mice at early stages of propagation secrete mainly the 3H9H/endogenous κ. These mAbs do not bind DNA as the κ chain is usually an efficient editor. That this combination prevails is not because Vκ4 cannot associate with 3H9 H chain, because hybridomas secrete the 3H9H/Vκ4 Ab at later stages of propagation when they have lost the endogenous κ. Thus, receptor editing is accomplished not by replacement of a Vκ gene, but by competition of L chains for association with the anti-DNA H chain.

Efficient phenotypic exclusion of the anti-DNA receptor can only succeed if the κ editor wins the competition for the H chain. Even the earliest studies of H/L association showed examples of H chain that strongly favored one L chain over others (23). Perhaps H/L combinations with complementary positive and negative charges such as basic anti-DNA VHs and acidic Vκ editors prefer each other. Even though efficient exclusion has two prerequisites, rearrangement of an editor and preferential association of the editor with H chain, this complex can lead to efficient editing of an autoreactive receptor (9).

Phenotypic exclusion may not be efficient when neither L chain is strongly preferred by the H chain. The relative frequency of the two receptors should correlate with the dimerization rate of either L chain for the H chain and could in principle lead to B cells with frequencies of the two receptors ranging from close to 100% to 50%. Such heterogeneity is seen in T cell populations that express both α alleles (for a review, see reference 24). Here we show the extreme case in which neither L chain is preferred over the other. The 3H9H/56R κ/λ B cells appear to have similar frequencies of κ and λ Ab on their surface (Fig. 2), although we do not know the molar amounts of either L chains. Nevertheless these partially autoreactive B cells appear to be harmless in vivo perhaps because they are diverted to the MZ. The mechanism by which these κ/λ B cells are directed to the MZ in unknown. Self-reactivity may be part of the reason, but other self-reactive B cells are excluded from follicles and remain in the outer T cell zone, where they die in 1–2 d (25). It is possible that both the affinity and the property of the self-antigen can play a role in determining the exact fate of self-reactive B cells (19).

How common are allelically or isotypically included B cells? κ/λ B cells have been noticed in normal (26) and transgenic mice (27) as well as human peripheral B cells (28). In mice carrying the transgene encoding for Ab against phosphocholine (PC), the autoreactive B cells that express the H and κ L chain transgenes coexpressed the endogenous λ L chain. The λ chain is believed to lower the anti-PC receptor density so that these B cells are rescued from deletion and anergy. In 3H9H/56R mice, however, both the κ and λ L chains are produced by endogenous rearrangement instead of transgenes. In addition, the combination of λ and 3H9/56R H chain binds DNA, while the coexpressed κ, such as 38c or 21-D is a good editor that vetoes DNA binding (10). As B cells can include κ and λ, it would seem that κ allelic inclusion might also be common. Such B cells might be overlooked because, unlike κ/λ, their phenotype is indistinguishable from allelically excluded cells using conventional anti-κ reagents. Isolated examples have been reported but studies with allele-specific reagents (7) and at the level of mRNAs in single cells (29) show the frequency of allelic inclusion to be extremely low. Thus, the high frequency of κ/λ B cells reported here for the 3H9H/56R transgenic is a special case. Nevertheless, this case may be helpful for thinking about the dynamics of tolerance by editing. A B cell that undergoes rearrangement goes through stages at which it decides whether or not to continue rearrangement. This decision is based on the functionality and specificity of the Ab coded for by the rearranged genes. The organization of the κ locus and the existence of multiple L chain loci maximize the steps that a B cell can take: a B cell with two κ chains, one of which contributes to autoreactivity, can continue to edit until the autoreactive κ gene is deleted. Depending on how many rearrangement attempts are needed for deletion, the B cell may extend rearrangement to the λ loci. In human, further editing can continue at λ while deletional rearrangments continue at the κ locus. As experiments on human B cells bear out, this process leads to few allelically and isotypically included B cells.

The mouse is exceptional because of its abbreviated λ locus. In the case of anti-DNA H chains, editing occurs only in the rare B cell that rearranges λx, and when the λ1 or λ2 (that sustain DNA binding) are expressed, they cannot be deleted. Thus, unlike human B cells, the transition to λ in the 3H9H/56R mouse almost always leads to a B cell that is irreversibly anti-DNA. The only salvation for such a B cell is to find a κ editor and such a partially autoreactive B cell, perhaps because of reduced density of the autoreactive receptor, is diverted to the MZ. Thus, the 3H9H/56R provides us with a model of the end stage of receptor editing. In human, this stage may rarely be reached because of the great variety of Vκ, Vλ, and J segments. But if editing is inappropriately initiated or sustained, as may be the case

in autoimmunity, then the 3H9H/56R mouse model may be instructive.

We thank Dr. John F. Kearney (University of Alabama at Birmingham) for critical reading of the manuscript.

This work is supported by National Institutes of Health grant AI-43532-02.

Submitted: 22 August 2001
Revised: 8 November 2001
Accepted: 21 November 2001

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