Receptor Editing: An Approach by Autoreactive B Cells to Escape Tolerance
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Summary
To determine the fate of anti-DNA antibody-bearing B cells in normal mice, we generated transgenic mice bearing the heavy (H) and light (L) chain genes of a well-characterized anti-double-stranded DNA antibody. This antibody was originally isolated from a diseased MRL/lpr mouse and has characteristics common to spontaneously arising anti-DNA antibodies. Results show that the H/L transgene (tg) immunoglobulin receptor is not expressed by animals bearing both tgs, although single tg animals (H or L) express their transgenes. Young H/L tg animals express few B cells, whereas adult H/L tg animals maintain almost normal B cell numbers. Analysis of the immunoglobulin receptors used by adult B cells shows that all contain the tg H chain in association with endogenous L chains. These B cells transcribe the L tg as well as the rearranged endogenous L chain gene, and loss of endogenous L chain gene transcription results in resurrection of the 3H9 H/L tg product. Examination of the endogenous L chains used by these cells shows that they represent a highly restricted subset of V genes. Taken together, these data suggest that autoreactive transgenic B cells can rearrange endogenous L chain genes to alter surface receptors. Those L chains that compete successfully with the L tg for H chain binding, and that create a nonautoreactive receptor, allow the B cell to escape deletion. We suggest that this receptor editing is a mechanism used by immature autoreactive B cells to escape tolerance.

B cells expressing autoreactive Ig receptors are thought to be negatively regulated through inactivation or deletion. Inactivation was observed by Pike and Nossal (reviewed in reference 1), who demonstrated that low doses of antigen could render B cells specifically unresponsive to antigen and mitogen stimulation. This type of tolerance was shown in amplified form using mice with transgenes (tg) coding for an anti-hen egg lysozyme (HEL) antibody (reviewed in reference 2). These mice, when crossed with mice with a tg coding for HEL, yielded double-tg progeny whose B cells were refractory to antigenic or mitogenic stimulation. Deletion was dramatically demonstrated using a tg system in which genes of an anti-H-2α antibody were introduced into mice of the H-2β haplotype (reviewed in reference 3). These mice showed a great reduction in B cell number and absence of the tg idiotype on the remaining peripheral B cells. These types of negative regulation operate at the cellular level through uncoupling signaling pathways or by initiating cell death. Another level at which regulation might occur is by changing the specificity of antiself receptors (4). Such "editing" might occur at the genetic level by replacing V genes coding for antiself receptors with V genes coding for "harmless" receptors. Mechanisms are available at both the H and L chain κ loci for V gene replacement. Here we describe another way of editing receptors. In this case, the L chain of an autoreactive antibody has been displaced by different L chains to yield nonautoreactive specificities. This form of editing occurs at the level of H/L pairing and depends upon the ability of a nonautoreactive L chain to displace an autoreactive L chain.

This phenomenon has been observed in a mouse with H and L tgs coding for an anti-DNA antibody. This antibody, termed 3H9, arose in a diseased MRL/lpr mouse and has the characteristics of autoantibodies associated with autoimmune disease: it binds single-stranded (ss)DNA, double-stranded (ds)DNA, and cardiolipin; it is a member of an expanded clone and is highly mutated to include at least one mutation that creates the specificity for dsDNA and cardiolipin (5, 6). We are unable to detect this antibody in tg animals. In addition, young tg mice exhibit greatly reduced numbers of B cells. Results indicate that the remaining B cells have altered their surface receptors through L chain competition. We pro-

†Abbreviations used in this paper: ANA, anti-nuclear antigen; ds, double stranded; tg, transgene.
pose that this receptor editing has saved these B cells from deletion.

Materials and Methods

Cloning of the V\kappa Gene. The hybridoma cell line 3H9 was used to clone the functionally rearranged V\kappa gene (5). The 9.4-kb BamHI fragment was isolated and cloned following a procedure by Carmack et al. (7). The purified insert was subcloned into the BamHI site of the pBS plasmid.

Production and Identification of tg Mice. Tg mice were generated and identified as previously described (7). Presence of the 9.4-kb BamHI fragment identified tg-positive animals. Seven tg founder mice were identified and backcrossed onto BALB/c to establish lines. Tg copy number was established for each founder by scanning Southern blots of BamHI-digested DNA probed with 32P-labeled Jk/Ck-specific pECK probe (8) using a two-dimensional proportional scanner (AMBIS, Inc., San Diego, CA). Total counts from transgene bands were compared with those of germline bands.

Hybridomas. Spleen cells were cultured for 2 d with Escherichia coli LPS (L2880; Sigma Chemical Co., St. Louis, MO) at a concentration of 20 \mu g/ml and then fused with either Sp2/0-Ag14 (9) or X63-Ag8 (10) cells as described (11). Cells were seeded into 96-well culture plates at limiting dilution. Hybridomas were selected for growth in azaserine-hypoxanthine (A9666; Sigma Chemical Co., St. Louis, MO) and 10% FCS. 1.209 or 1.3H9 antibodies were then added to wells containing anti-K antibody, 1.209, 1.3H9, or no primary antibody, followed by incubation with a biotinylated goat anti-mouse IgG1 reagent (Fisher Biotech), then incubation with Texas red-conjugated avidin (Molecular Probes, Inc., Eugene, OR). Spleen and bone marrow samples were counterstained with FITC-coupled goat anti-mouse IgM (Fisher Biotech). 3H9 and 104.8 hybridomas (see Fig. 4) were incubated with FITC-coupled anti-mouse IgG2b (Fisher Biotech). Propidium iodide was used to exclude dead cells from analysis. Analyses were performed using a dual-laser FACStar Plus® (Becton Dickinson & Co., Mountain View, CA).

Results

Construction of the V\kappa4 Fragment and Its Expression in tg Mice. The V\kappa4Jk4Ck gene expressed by the 3H9 hybridoma was cloned as a 9.4-kb BamHI fragment (Fig. 1 a). The V\kappa exon of this clone was sequenced and found to be identical to the V\kappa cDNA sequence reported for the 3H9 \kappa chain (5). In addition, cotransfection of the V\kappa4 construct with a 3H9 H chain construct into non-Ig-secreting cells led to secretion of an antibody with the specificity of 3H9 (data not shown).

Tg mice were generated using this V\kappa4 construct. Fig. 1 b shows Southern analysis of tail DNA from the founder selected for all subsequent experiments. The predominant 9.4-kb band corresponded to the introduced V\kappa4 fragment. Additional bands in the high molecular weight region and ~9.4 kb suggested that some tg copies had modified their BamHI ends. Scanning of blots with a two-dimensional proportional scanner and comparison of tg bands to germline bands showed this founder to carry 20–40 tg copies. Transcriptional analysis showed tg expression in bone marrow, spleen, and at very low levels in thymus. No tg transcripts were detected in heart, brain, lung, or liver (data not shown).

Endogenous L Chain Expression in L tg Mice. A key property of an Ig H or L tg is its ability to shut down endogenous Ig gene rearrangement. We analyzed the extent of endogenous L chain gene rearrangement and expression in hybridomas derived from LPS-activated spleen cells of V\kappa4 L tg animals. Ig-secreting hybridomas were examined for unique \kappa rearrangements by Southern blot analysis. As shown in Fig. 1 b, DNA from these hybridomas exhibited the characteristic tg pattern as well as bands corresponding to \kappa genes from the fusion partner SP2/0. Unique bands representing endogenous rearrangements could be observed in certain hybrids (2748.9.1 and 2748.14.1; Fig. 1 b), whereas another (2748.10.1) exhibited no additional rearrangements. This latter case suggested that the L tg does exclude endogenous \kappa rearrangements in some B cells.
Figure 1. (a) The 3H9 L tg construct. (b) Southern blot analysis of L tg tail, L, and H/L tg splenic hybridomas. BamHI-digested DNAs from L tg tail, BALB/c liver, Sp2/0, 3H9, L tg splenic hybridomas 2748.9.1, 2748.10.1, 2748.14.1, and H/L tg splenic hybrids 2754.21.1 and 2754.21.3 were examined by Southern analysis as described in Materials and Methods. In addition to the hybridization pattern observed for all L tg-containing hybridomas, unique bands representing endogenous K rearrangements were seen in 2748.9.1, 2748.14.1, 2754.21.1, and 2754.21.3; these bands are marked by adjacent arrows.

It was possible that additional endogenous rearrangements might be hidden in the complex Southern blot patterns of the tg hybridomas. As an alternative approach to determine the influence of the L tg on endogenous L chain expression, we tested these hybridomas for transcription of endogenous K genes. Assays using primers specific for JK1, JK2, JK4, JK5, M, and X2 showed that 19 of 25 hybrids derived from L tg animals transcribed an endogenous L chain transcript (Table 1). Fig. 2 A shows results using the JK1-specific primer. The lower band in each lane of Fig. 2 A corresponded to the cDNA extension product of the aberrant SP2/0 K transcript found in all SP2/0-fused hybrids. In addition, hybridoma 2748.7.1 generated a second product presumably corresponding to an endogenously derived K transcript. Hybridomas 2748.10.1 and 2748.5.3 did not show a product using the JK1 primer (Fig. 2 A), or other primers (data not shown), and are presumed to lack endogenous L chain transcripts.

Extension analysis using the L tg-specific primer showed that the tg was transcriptionally active in all 25 L tg hybrids (Fig. 2 A, and data not shown). Those hybrids transcribing only the L tg also secreted k-containing Ig, indicating that the L tg can produce a functional protein.

Cytofluorographic Analysis of tg Animals. We followed expression of the 3H9 antibody in 3H9 H/L tg animals using two antiidiotypic mAbs. The specificities of these reagents are demonstrated in Fig. 3. 1.209 bound surface 3H9 Ig on 3H9 hybridoma cells and surface Ig composed of the 3H9 H chain in association with a Vκ8 L chain on 104.8 cells. Because Vκ4 and Vκ8 L chains are only distantly related, these data suggested that the idiotope bound by 1.209 is on the H chain. Further analysis showed that 1.209 bound other 3H9H chain–containing antibodies associated with a wide variety of L chains (J. Erikson, Wistar Institute, Philadelphia, PA, unpublished results). 1.3H9 bound 3H9 antibody, but this reagent failed to bind other antibodies using either 3H9 H or L chain in association with unrelated chains. We presume that 1.3H9 is specific for a 3H9H/L combinatorial idiotope.

We analyzed day 4 and adult tg mice by cytofluorography using these antiidiotypes in conjunction with an anti-IgM reagent (Fig. 4). The anti-3H9 H chain reagent, 1.209, bound 50–60% of day 4 splenic and adult bone marrow IgM + cells and 90% of adult splenic IgM + cells from H tg animals and H/L tg animals (Fig. 4 a). The antiidiotypic reagent 1.3H9 failed to stain cells from any population tested (Fig. 4 b). Most importantly, this reagent bound neither cells from the adult nor cells from the day 4 H/L tg animals.

The above data suggested that 3H9-bearing B cells are deleted in these animals. Deleting tg mouse models have shown that loss of the autoreactive tg specificity is accompanied by profound B cell depletion (3, 19, 20). The number of IgM + B cells from day 4 H/L tg animals was greatly reduced compared with that of L tg or normal animals. The H tg animal showed less severe depletion. In contrast, bone marrow and spleen of adult H tg and H/L tg animals had near normal numbers of B cells.

Hybridomas from 3H9 H/L tg Mice. Hybridomas derived from LPS-activated splenic B cells of H/L tg animals and their littersmates were examined for expression of the 3H9 idiotope using both antiidiotypic reagents 1.209 and 1.3H9. In addition, each Ig was assayed for ANA staining of fixed Hep-2 cells to look for the homogeneous nuclear staining pattern characteristic of 3H9. By these criteria, none of the 228 hybridomas produced an Ig with the properties of 3H9 (Table 2). Three H/L tg hybrids secreted antibody that produced a 3H9-like ANA pattern; however, none of these had the 3H9 idiotope, nor did they bind DNA. These three antibodies did bind to isolated histones (M. Monestier, Center for Molecular Medicine and Immunology, Newark, NJ, unpublished results), perhaps explaining their ANA pattern. 2 of 42 hybrids from L tg animals exhibited the 3H9 idiotope, but did not bind DNA or stain nuclei of HEP-2 cells. The L tg product in these hybrids may be associated with a nonautoreactive 3H9-like H chain.
Although none of the antibodies from H/L tg hybridomas expressed the 3H9 idiotope, most had the 3H9 H chain as determined by binding to the 1.209 reagent. Sequencing of H tg mRNA from numerous H/L tg hybridomas revealed no V region mutations; therefore, failure to express the 3H9 idiotope seemed likely to result from lack of surface expression of the L tg product. This might result from impairment of the L tg or from 3H9 H chain association with endogenous L chains. To assay L tg expression as well as any endogenous L chain gene expression, primer extension analysis was undertaken on 19 H/L hybrids. As summarized in Table 1, all analyzed H/L tg hybrids transcribed an endogenous \( \kappa \) mRNA as well as the L tg. Sequencing of the L tg transcript from several of these hybrids revealed no V region mutations. Therefore, the presence of endogenous L chains rather than impairment of the L tg appeared to prevent expression of the H/L product in these hybrids.

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The fact that 3H9 was undetectable indicated that either L tg expression was greatly reduced or that endogenous L chains successfully competed for 3H9 H chain binding in these hybrids. Relative levels of endogenous and tg L chain mRNA were compared by primer extension analysis using IgM H chain mRNA as an internal standard. Fig. 2 B shows results for two H/L tg hybrids, and Table 3 summarizes results for 20 hybrids. 10 of these hybrids expressed approximately equal or higher relative levels of tg mRNA than endogenous \( \kappa \) mRNA, indicating that in these hybrids at least, lack of 3H9 expression was not due to deficient L tg expression.

Restriction in the number of different endogenous L chains used was suggested by the fact that none of the antibodies from the H/L hybrids exhibited specificity for dsDNA despite previous work, which had shown that the 3H9H chain could associate with a wide variety of L chains to create this specificity (12). Sequence analysis of endogenous \( \kappa \) chains from

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### Table 1. Endogenous L Chains Used by L tg and H/L tg Hybridomas (continued)

| Hybrid    | \( V_k \)   | \( J_k \)   |
|-----------|-------------|-------------|
| H/L tg:   |             |             |
| 2754.1.1  | \( V_k12-13^* \) | \( J_k2 \)  |
| 2754.3.2  | \( V_k12-13 \)  | \( J_k1 \)  |
| 2754.5.5  | \( V_k12-13 \)  | \( J_k1 \)  |
| 2754.14   | \( V_k12-13 \)  | \( J_k1 \)  |
| 2754.17.8.4 | \( V_k12-13 \) | \( J_k1 \)  |
| 2754.19   | \( V_k12-13 \)  | \( J_k4 \)  |
| 2664.1    | \( V_k12-13^* \) | \( J_k2 \)  |
| 2664.5    | \( V_k12-13 \)  | \( J_k4 \)  |
| 2664bm4   | \( V_k12-13 \)  | \( J_k5 \)  |
| 2664bm2   | \( V_k12-13^* \) | \( J_k2 \)  |
| 2754.21.3 | \( V_k21 \)    | \( J_k1 \)  |
| 2754.20   | \( V_k1 \)     | \( J_k4 \)  |
| 2754.17.6 | \( V_k19 \)    | \( J_k4 \)  |
| 2664bm5   | \( V_k19 \)    | \( J_k4 \)  |
| 2754.15.2 | \( V_k38C \)   | \( J_k4 \)  |
| 2664.6    | \( V_k38C \)   | \( J_k1 \)  |
| 2754.13.2 | \( V_k5 \)     | \( J_k5 \)  |
| 2754.2.2  | \( V_k5 \)     | \( J_k5 \)  |
| 2664.4    | \( V_k9 \)     | \( J_k1 \)  |

\( J_k \) use was determined by primer extension analysis of hybridoma mRNA (see Fig. 2). Endogenous L chains were presumed to be in germline configuration when only tg mRNA could be detected. L chain mRNA sequencing was carried out using the appropriate \( J_k \) primer as described in Materials and Methods. Family identity was determined using the \( V_k \) gene classification given by Strohal et al. (41).

\( ^* \) mRNA extension gel patterns proved highly characteristic for particular VK products. Pattern comparisons of such extensions provided assignment of \( V_k \) genes to unsequenced mRNAs.
H/L tg hybridomas showed that, indeed, these antibodies expressed a very limited set of Vκ genes (Table 1). 10 of 19 antibodies from H/L tg hybrids used members of the Vκ12,13 group. Sequence analysis also indicated that Vκ groups 5, 19, and 38c were overrepresented.

In addition to overrepresentation of a few groups, a high degree of homology was noted between members of each group, suggesting use of one or several highly related members. Thus, Vκ12,13 sequences were 93–98% homologous (sequence ambiguities prevented more conclusive determination of homology). A high degree of homology (at least 96%) was also observed between the Vκ5 genes used by hybrids 2754.13.2 and 2754.2.2, suggesting that both originated from one member of this family. Vκ19 genes used by 2754.17.6 and 2664 bm5 were ~93% homologous, again suggesting use of several highly related members. Clonal relatedness among hybrids using similar L chains was disproved by examination of endogenous H chain rearrangements (data not shown).

**Generation of Endogenous L Chain Loss H/L Hybrids That Express the 3H9 Phenotype.** In subcloning H/L hybrids, variants were isolated that had lost endogenous κ mRNA transcription. Fig. 1 shows Southern analysis of one subclone,
Figure 4 Cytofluorographic analysis of day 4 splenic and adult splenic and bone marrow B cells from tg animals using anti-3H9 monoclonal reagents. Spleen and bone marrow cells from animals carrying the L tg, the H tg, or both H and L tgs were costained with FITC-coupled anti-mouse IgM and (a) 1.209 or (b) 1.3H9 (revealed by Texas red-avidin binding) as described in Materials and Methods and analyzed by cytofluorography. Non-tg littermates had similar cytofluorographic patterns as L tg animals (data not shown). Results from four experiments analyzing day 4 mice and from eight experiments analyzing adult mice showed similar profiles.

Table 2. Occurrence of the 3H9 Phenotype in Antibodies from Splenic Hybridomas

| Hybrids from: | IgM* | Antibodies binding antiidiotypes | Antibodies exhibiting 3H9 ANA pattern | Antibodies exhibiting 3H9 phenotype |
|---------------|------|-------------------------------|-------------------------------------|-----------------------------------|
| Splenic Hybridomas |  | 1.3H9 | 1.209 | 3H9 ANA pattern | 3H9 phenotype |
| H/L spleen | 72/72 | 0/72 (0) | 62/72 (86) | 3 | 0 |
| H spleen | 56/56 | 0/56 (0) | 56/56 (100) | 0 | 0 |
| L spleen | 42/42 | 2/42 (5) | 2/42 (5) | 0 | 0 |
| –/– spleen* | 58/58 | 0/58 (0) | 0/58 (0) | 0 | 0 |

All splenic hybridomas secreted antibody of the IgM isotype. Antibody was assayed for binding to antidiotypic reagents and to HEP-2 cells (ANA) as described in Materials and Methods.

* Number of animals represented from each group are: –/– spleen, two; L tg spleen, four; H tg spleen, two; H/L tg spleen, three.
Table 3.  Relative Levels of Endogenous and Tg L Chain mRNA in H/L Hybridomas

| Hybrid     | Endogenous L chain mRNA*/L Tg mRNA |
|------------|-----------------------------------|
| 2754.1.1   | 20.9                              |
| 5171.2     | 14.2                              |
| 5171.6     | 9.3                               |
| 2754.15.2  | 7.2                               |
| 2664BM5    | 5.7                               |
| 2664BM2    | 3.4                               |
| 2754.20    | 3.4                               |
| 2664.1     | 3.4                               |
| 2754.14    | 2.3                               |
| 5171.4     | 1.7                               |
| 2754.5.5   | 1.4                               |
| 2754.21.3  | 1.3                               |
| 2754.3.2   | 1.2                               |
| 2754.19    | 1.1                               |
| 2754.13.2  | 0.6                               |
| 2754.17.8.4 | 0.6                           |
| 2754.2.2   | 0.4                               |
| 2664.5     | 0.4                               |
| 5171.1     | 0.1                               |

* Relative levels of L chain mRNA were quantitated by comparing radioactive counts from L chain cDNA bands to radioactive counts from an internal standard, IgM H chain cDNA. See Fig. 2 B for details. The ratio of counts of endogenous L chain or L tg-specific product to counts of IgM H chain-specific product, was taken as a relative concentration of L chain mRNA. The ratio of the relative amount of endogenous L chain mRNA to the relative amount of L tg mRNA is shown above.

2754.21.1, that had lost endogenous L chain transcription, and a sibling, 2754.21.3, that retained endogenous L chain transcription. They exhibited an identical banding pattern indicating that both arose from a single progenitor. Primer extension analysis showed that both transcribed the L tg, but that subclone 2754.21.3 transcribed an endogenous κ mRNA whereas 2754.21.1 did not (Fig. 2 A). Loss of endogenous κ transcription might be due to a mutation in the observed rearrangement (see Fig. 1) or by loss of a κ gene not discernible by this Southern analysis.

FACS® analysis of these hybrids showed that antibody on both bound the anti-3H9 H chain antibody 1.209 and thus expressed the 3H9 H chain (Fig. 5 a). However, 2754.21.1 also bound the antiidiotypic reagent 1.3H9 (Fig. 5 b), and produced a homogeneous nuclear pattern on fixed Hep-2 cells (data not shown). According to the above-described criteria, this cell expressed the 3H9 antibody.

Discussion

We are interested in understanding how anti-DNA antibodies, arising in diseased MRL/lpr mice, are negatively regu-
by as much as 75% as compared with L tg littermates. H
tg mice also frequently bore fewer B cells presumably reflecting
the dominant role of the 3H9 H chain in creating autoreactive
specificities. Although these data suggest that anti-dsDNA
antibody-bearing B cells are deleted in young animals, we
cannot exclude the possibility that these lower numbers reflect
abnormal development of B cells in tg animals.

To determine the site of regulation, we tested the bone
marrow B cells of H/L tg mice for expression of the 3H9
idiotope. In contrast to other autoantibody tg models (3, 4,
19, 20), we could not detect 3H9 antibody. This is not due
to competition for antiidiotype binding by self-antigen, as
this antiidiotype is not ligand (DNA) inhibited (data not
shown). Instead, this result suggests that regulation of these
B cells must occur at the initial stages of surface expression.

Even though the majority of B cells in H/L neonates are
deleted, significant numbers remain, and adult tg mice have
near normal B cell numbers. These B cells express altered
receptors that retain the H chain idiotope but not the L/H
idiotope. Since the 3H9 idiotope requires both 3H9 H and
L chain, this shows that the L tg is not expressed on the
surface of these cells. Hybridomas from H/L animals exhibit
the same phenotype as splenic B cells. Transcriptional anal-
ysis shows that all hybridomas examined transcribe the L tg
as well as an endogenous k gene. We think it unlikely that
the L tg transcript is untranslated. Subclones of several hy-
bridomas yield variants that have lost the endogenous k tran-
script but have retained the tg transcript. Such variants now
express the tg L chain-dependent 3H9 idiotope. We believe
that the loss of expression of the 3H9 idiotope must be due
to displacement of the 3H9 L chain by an endogenous L chain.

For the 3H9 receptor to change to a nonautoreactive
receptor, three requirements must be met.

(a) The tg-encoded Antibody Must Permit Rearrangement
of Endogenous L Chain Genes. Considerable evidence indicates
that TCR-\(\alpha\) and Ig L chain gene rearrangement can con-
tinue after functional rearrangement and even receptor ex-
pression on T and B cells. Sequential rearrangement on the
same allele to replace existing functional rearrangements and
rearrangement on both alleles to generate two functional prod-
ucts have been demonstrated (22–27). Also, expression of
recombinase-associated genes (RAG-1 and RAG-2) has been
observed in immature thymocytes and B cells bearing surface
receptors (4, 28). In apparent contradiction to these findings,
numerous groups have shown that expression of a tg anti-
body in Ig Ltg mice or expression of a tg TCR in \(\alpha/\beta\) tg
mice can lead to shutdown of endogenous rearrangement (29,
30). To reconcile these contrary findings, several groups have
suggested that expression of selected receptors does shut down
rearrangement, whereas expression of inappropriate or un-
selected receptors may not (4, 31). In the 3H9 tg model, we
cannot distinguish between the possibilities that 3H9, as an
autoreactive receptor, may actively promote rearrangement
to encourage receptor editing, or that tg expression may not
shut down rearrangement.

(b) The Endogenous L Chain Repertoire Must Include Members
That Change 3H9 Specificity. Since the result of receptor al-
teration is to shift specificity from self to nonself, the L chain
repertoire must include L chains that modify or change 3H9
specificity. This is bound to be a restricted set in view of
the variety of L chains that sustain an anti-dsDNA specificity
in combination with 3H9H (12). Indeed, sequence analysis
of endogenous V\(x\) genes by these H/L hybrids shows con-
siderable restriction in the genes selected (Table 1). Most strik-
ingly, single members of the V\(x\)5, 12, and 19 families were
repeatedly selected by B cells from two different H/L mice.
In addition, comparison of V\(x\) genes used by H/L hybrids
and those used by H hybrids shows considerable overlap (32).
Highly homologous genes from V\(x\)1, 5, 9, 12, and 19 families
are used by both H and H/L B cells, reflecting a common
requirement to create nonautoreactive phenotypes with a fixed
H chain (3H9H). In H/L cells, these L chains fulfill the added
requirement of successfully competing with the L tg product
for H chain binding. Other V\(x\) genes, observed in H hybrids
have not been found in H/L B cells. These include members
of the V\(x\)8, 2, 9A, and 21 families. The restricted use of these
genes by H but not H/L B cells may simply result from vari-
ability in L chain use by different animals. Alternatively, it
may reflect the inability of these gene products to compete
with the 3H9 L chain in H/L B cells.

(c) Certain Endogenous L Chains Must Be Favored over the
3H9 L Chain. As shown above, we were unable to detect
the 3H9 idiotope on either tg B cells or among secreted anti-
bodies. We explain this by L chain replacement. Since we
assume that the antiidiotope is reasonably sensitive, this re-
sult implies that the 3H9 H chain usually associates with
endogenous L chain protein. This finding cannot be explained
by an overabundance of endogenous L chain mRNA. As ex-
plained in Table 3, the ratio of endogenous L chain mRNA
to tg mRNA varies, and sometimes tg mRNA levels far ex-
ceed those of endogenous L chain levels. Although the reason
for this variation is not understood, the lack of tg L chains
on H/L hybrids and presumably B cells is not due to a shortage
of mRNA. One explanation for the preferential use of en-
dogenous L chains is that they outcompete tg L chains for
association with the 3H9 H chain. Preferences in H/L pairing
have been seen in competition studies carried out either in the
test tube or through hybridoma-hybridoma fusions (33–39).
Often, the homologous (original) H/L combination prevails, but numerous examples have been reported in
which an H chain prefers a heterologous (foreign) L chain
(33–39). The latter case could explain our findings. It is, how-
ever, surprising that heterologous L chains are so strongly
preferred by the 3H9 H chain in H/L hybrids, as evidenced by
the fact that we do not observe even low concentrations of
3H9 on these cells. Potentially, the deletion process may be
sensitive enough to get rid of such low expressors. Addi-
tionally, the 3H9 H/L association may not be particularly
strong. The examples in the literature that have shown
preferential homologous reassociation have undergone affinity
maturation and may also have evolved better H/L associa-
tion. Since our example is an autoantibody, the selection forces
may be quite different. It may be of interest to carry out H/L
reconstitution experiments with antibodies that have not been
under positive selection.

Tieg et al. (4) have reported similar findings in "centrally

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deleting” anti-H-2k antibody tg-bearing, H-2k-positive mice. Residual B cells found in the periphery of these animals are idiootype negative. In agreement with our results, these cells express the H tg product in association with some endogenous L chain (frequently λ) that apparently competes with the L tg for H chain association. However, the anti-H-2k tg system promotes more severe, long-term depletion of peripheral B cells, whereas the 3H9 tg system appears to provide a better environment for endogenous L chain gene rearrangement and subsequent escape from the deleting process. This difference most likely reflects an increased efficiency by the anti-H-2k tgs to shut down endogenous rearrangement. Lack of the downstream enhancer in the 3H9 L tg construct may decrease its ability to exclude endogenous rearrangement (40). Conversely, in the anti-H-2k tg system, cointegration of H and L tgs into one location might increase L tg expression through use of H gene enhancer and/or facilitate coordinated expression of H and L tgs. Either of these events might provide this system with a better means of suppressing endogenous gene expression.

The concept of receptor editing implies that recombinase activity is reactivated in immature autoreactive B lymphocytes. Given recombinase reactivation, an edited cell must often pass through a stage of dual L chain expression (an exception to this is editing by V gene replacement, yet even here the cell can also rearrange on the “other” κ allele or at the λ locus). Dual L chain expression may commonly lead to death. Perhaps the autoreactive receptor is not sufficiently diluted to escape deletion, or the density of any given receptor is too low to elicit positive selection. For those cases where the H chain favors one L chain and this H/L pair is not autoreactive, preferential pairing may be an important editing mechanism. On the other hand, V gene replacement that lacks the potential problems of dual L chain expression may also be observed in mature cells. In either case, the data presented in this paper suggest that receptor alteration through continued L chain gene rearrangement may be a valuable means of salvaging autoreactive B cells.

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