B Lymphocytes May Escape Tolerance by Revising Their Antigen Receptors

By Marko Z. Radic,* Jan Erikson,† Samuel Litwin, and Martin Weigert

From the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111; the *Department of Microbiology and Immunology, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129; and †Wistar Institute, Philadelphia, Pennsylvania 19104

Summary

To explore mechanisms that prevent autoreactivity in nonautoimmune mice, endogenous immunoglobulin (Ig) light (L) chains that associate with a transgenic anti-DNA heavy chain were analyzed. The antibodies from splenic B cell hybridomas of such mice did not bind double-stranded DNA (dsDNA) and their L chain sequences showed a biased use of V~ and J~ gene segments. The 44 L chains in this survey were coded for by just 18 germline genes. Six of the genes, each belonging to a different V~ group, were used more than once and accounted for three fourths of all sequences. Based on the distribution of V~ genes, the L chain repertoire in this line of transgenic mice was estimated at 37 V~ genes. The most frequently observed gene, a member of the V~ 12/13 group, was identified in 16 hybrids. In addition, the majority of V~ genes used J~5. We interpret the skewed representation of V~ and J~ gene segments to result from negative selection. Based on the data, we suggest that V~ rearrangements giving rise to anti-dsDNA reactivity are removed from the repertoire by a corrective mechanism capable of editing self-reactive Ig.

Tolerance to self has been studied in Ig transgenic models of autoreactivity. By using Ig transgenes against facultative self-antigens, it has been shown that self-reactive B cells are selected against by anergy or deletion (1, 2). We have constructed mice with transgenes that code for anti-DNA antibodies and obtained similar results. The majority of splenic B lymphocytes from mice with the V~,3H9 and V~,8 genes express anti-single-stranded DNA (ssDNA)1 antibodies on their surface, but we do not detect them in the serum (3). By analogy to Goodnow et al. (1), anti-ssDNA B cells in those mice appear to be anergic.

Analysis of splenic B cells from mice containing just the V~,3H9 transgene suggested a second feature of tolerance to DNA. No anti-double-stranded DNA (dsDNA) activity was detected among hybridomas from these mice. This was surprising, since previous studies have established that the 3H9 H chain can combine with a diverse range of L chains to yield antibodies capable of binding to both ssDNA and dsDNA (4, 5). The absence of hybrids producing anti-dsDNA suggested that dsDNA-specific B cells are functionally deleted. Nevertheless, V~,3H9 mice have near normal numbers of splenic B cells, and hybridomas can be readily obtained (3). Some of the antibodies produced by these hybridomas bound ssDNA, whereas others did not bind DNA at all. We have now examined the V~ and J~ gene segment use of hybrids obtained from two of these mice. The mAbs were characterized by a sharply reduced repertoire of L chains. L chains that are found among spontaneous anti-DNA antibodies from MLR/lpr (4) or NZB × NZW (6) mice were absent from our sample. In addition, J~5 was overutilized. The data suggested that the major driving force shaping the L chain use in V~,3H9 mice is selection against dsDNA-binding B cells. Furthermore, the data are consistent with the notion that B cells can escape deletion if their autoreactive surface receptors are replaced through further rearrangements.

Materials and Methods

Hybridoma Fusions. B cell hybridomas were generated from the spleens of transgenic V~,3H9 mice maintained in our animal colony. A 3-mo-old female mouse (No. 104) was injected intraperitoneally with 50 µg LPS as described (3), and its spleen cells fused to the SP2/0 fusion partner 3 d later. The second fusion was carried out using splenic B cells from a 4-mo-old male V~,3H9 mouse (No. 2352) which were sorted for the presence of the B220 and V~ surface markers by FACS® (Becton Dickinson & Co., Mountain View, CA). B cells from the second mouse were stimu-

1 Abbreviations used in this paper: dsDNA, double-stranded DNA; MLE, maximum likelihood estimate; rp, reciprocal products; ssDNA, single-stranded DNA.
lated in vitro with 30 μg/ml LPS before fusion. Hybridomas were selected using hypoxanthine–azaserine. Supernatants were tested for antibody production and H and L chain isotypes after colonies had grown sufficiently. Randomly selected hybridomas were expanded for further analysis.

**Extensions of Ig mRNA.** Poly(A)^+ mRNA was isolated from 42 hybridomas (picked to be representative of both fusions) using the method of Badley et al. (7) and oligo-dT cellulose (Collaborative Research, Bedford, MA). Approximately 3 μg of poly(A)^+ RNA was extended into cDNA using avian reverse transcriptase (Life Sciences, Inc., St. Petersburg, FL) and T4 polynucleotide kinase–labeled oligonucleotides complementary to the sense strand of each of the four functional J_1 segments. Since the SP2/0 fusion partner contains sterile transcripts from an out-of-frame V_21E-J_2 rearrangement, J_2 oligo (5'-CCCTCCGAACGTGTAAGG-Y) extensions from each hybrid were compared with the products from SP2/0. Authentic V_1-J_2 extension products could readily be detected using that approach.

The J_4 (5'-TATTTCCAACTTTGTCCCCGAG-Y) and J_5 (5'-GAAGCTCCAGTTGCTCCAGCAC-3') oligos that were used did not crosshybridize with other J_1 segments or with each other, thus allowing unambiguous assignments. In contrast, it was found that the J_1 oligo (5'-TGATTTCCAGCTTCCTGCCTC-CAC-3') was capable of extending mRNA starting from J_1 and J_2 sequences. Therefore, unambiguous J_1 or J_2 assignments were made after sequencing cDNAs obtained by reverse transcription from an end-labeled C_4-specific oligo and gel purification of full size extension products from denaturing PAGE (4).

**Sequence Analysis.** All rearrangements to J_1 or J_2 were sequenced by chemical degradation of cDNAs obtained by reverse transcription with a C_4-specific oligo, as described (8). Rearrangements to J_4 or J_5 were sequenced by a direct oligonucleotide-primed dideoxy chain termination method described by Geliebter et al. (9).

**Southern Blotting.** Genomic DNA was purified from hybridomas and digested with BamHI for L chain analysis or EcoRI for H chain analysis. DNA was run on a 0.8% agarose gel in Tris/acetate/EDTA buffer and transferred to nylon membranes (Zeta Probe; Bio Rad Laboratories, Hercules, CA) in 0.4 N NaOH as described by Reed and Mann (10). Three DNA fragments were labeled by random priming and used as probes for hybridizations. The probe used to detect H chain rearrangements was pJ11, a fragment spanning the H chain enhancer and activation of endogenous V_H genes. Since all hybrids in our collection produced only IgM antibodies, cDNA extension experiments were carried out using a C_μ oligonucleotide. None of the hybrids analyzed expressed an endogenous IgM mRNA. Instead, only the V_3H9-specific extension products were detected (data not shown). Moreover, the sequence of the transgene message from four hybrids showed no mutations. Therefore, the loss of dsDNA specificity in some hybrids and the complete loss of DNA binding in others must be due to the L chain. Although 12 of the V_4 genes identified here were observed only once, the remaining six occurred two or more times and together accounted for 32 of the 44 sequences (Table 1). Although some antibodies from V_3H9 mice bound to ssDNA with affinities that were comparable with antibodies from autoimmune mice, none bound dsDNA (data not shown).

These L chains have an unusual distribution of V_4 genes. Only nine of the more than twenty V_4 groups were observed. Moreover, the representation of V_4 groups that were observed was not proportional to their size. In addition to the disproportionate representation of certain V_4 groups, recurrent V_4 genes also characterized this sample. Although 12 of the V_4 genes identified here were observed only once, the remaining six occurred two or more times and together accounted for 32 of the 44 sequences (Table 1). The distribution of J_4 genes is also unusual in that J_5 accounted for the majority (43%) of the rearrangements observed (Table 1). This is different from J_1 gene segment frequencies in splenic B cells of nontransgenic mice. Various investigators have estimated that between 35 and 47% of V_4 genes rearrange to J_1, whereas only 6–25% use J_5 (15, 16). We compared the frequencies of all four J_4 segments in our sample with published values using a two by four contingency table and a generalized hypergeometric test (STATXACT, 17). According to this method, our data were significantly different from the data obtained by analyzing J_4 usage in LPS-treated splenic B cells of BALB/c mice described above (p <0.008).

Most of the J_3 segments (17 of 19) were associated with V_4 genes that were isolated more than once in our survey (Fig. 1), whereas J_4 use in single V_4 isolates favored J_1 (9

1166  V_4 Editing in Anti-DNA Transgenic Mice
Table 1. Summary of V\(_k\) and J\(_k\) Segments Used in 42 Hybridomas from V\(_k\)3H9 Mice

| V\(_k\) Group | V\(_k\) Gene | J\(_k\)1 | J\(_k\)2 | J\(_k\)4 | J\(_k\)5 | Total | ssDNA | MLE |
|---------------|-------------|--------|--------|--------|--------|-------|-------|-----|
| 1 (4–6)       | 1A          | 1      | –      | –      | –      | 1     | –     | 1   |
|               | 1B          | –      | 1      | 2      | –      | 3     | –     |     |
| 4/5 (25–50)   | 5A          | 1      | –      | –      | –      | 1     | –     |     |
|               | 5B          | –      | 1      | –      | 6      | 7*    | +     | 37* |
| 8 (5–16)      | 8A          | 1      | –      | –      | –      | 1     | 2     |     |
|               | 8B          | 1      | –      | –      | –      | 1     | –     |     |
| 9 (6–11)      | 9A          | 1      | –      | –      | –      | 1     | –     |     |
|               | 9B          | 1      | –      | –      | –      | 1     | –     |     |
|               | 9C          | 1      | –      | –      | –      | 1     | –     |     |
| 12/13 (2–8)   | 12A         | 2      | 4      | –      | 10     | 16*   | –     |     |
|               | 12B         | 1      | –      | –      | –      | 1     | –     |     |
| 19 (4–6)      | 19A         | 2      | –      | –      | –      | 2     | +     |     |
|               | 19B         | 1      | –      | –      | –      | 1     | –     |     |
| 21 (6–13)     | 21A         | 1      | –      | –      | –      | 1     | –     |     |
|               | 21D         | 1      | –      | 1      | –      | 1     | –     |     |
|               | 21I         | –      | –      | 1      | –      | 1     | –     |     |
| 34 (2–3)      | 34C         | –      | –      | –      | 1      | 1     | –     |     |
| New           | G4          | –      | –      | –      | 1      | 1     | –     |     |
| Total         | 15          | 6      | 4      | 19     |        | 44    | –     |     |

Hybridomas were grouped according to the V\(_k\) gene used. V\(_k\) genes were assigned to a V\(_k\) group and distinguished by a capital letter suffix. In keeping with this nomenclature, a new member of the V\(_k21\) group is called V\(_k21I\). Repeats of each V\(_k\) gene, numbers of rearrangements to each of the four J\(_k\) segments, and ssDNA affinities were indicated. Two hybridomas, 2352-37 and 2352-46, expressed two in-frame V\(_k\) rearrangements, thus 42 hybrids yielded 44 L chain sequences. One V\(_k\) gene, 1G1, belonging to an as yet unclassified V\(_k\) group (43), was the closest relative of the new gene listed here. MLE of the total L chain repertoire was calculated both with and without including the V\(_kSB\) and V\(_k12A\) genes. In the presence of those two most frequently used V\(_k\), the MLE predicted a repertoire of 20–25 V\(_k\) genes. A more conservative calculation of the MLE is indicated by an asterisk and did not include these two genes. Therefore, those two V\(_k\) genes were simply added to the MLE, bringing the total to 37 and the 90% confidence bounds to 23 and 87. By either measure, the extrapolated L chain repertoire in V\(_k3H9\) mice was much less than the potential germline repertoire of \(~200\) V\(_k\) genes.

Figure 1. J\(_k\) segments associated with single and recurrent V\(_k\) genes. The number of rearrangements to each of the J\(_k\) segments was plotted for V\(_k\) genes seen only once in this survey (darker bar) and V\(_k\) genes that were seen at least twice (lighter bar).

of 12 examples). Overall, the J\(_k\) use in single isolates and repeats was significantly different (p \(<0.0042\)). Thus, the recurrent V\(_k\) genes accounted for the overutilization of J\(_k5\). Two of the recurrent genes, V\(_k12A\) and V\(_kSB\), accounted for 16 J\(_k5\) rearrangements, although both were also seen to associate with other J\(_k\) segments.

Independent Origin of Recurrent V\(_k\)-J\(_k\) Pairs. Several of the hybrids from either fusion expressed L chains composed of the same V\(_k\) and J\(_k\) gene segments. One possible reason for such recurrent V\(_k\)-J\(_k\) combinations among a set of hybrids is that they are derived from members of an expanded B cell clone. This possibility was tested by Southern blots of H and L chain rearrangements (Fig. 2).

Representative hybridization data from hybrids using V\(_k12A\) are shown in Fig. 2. Fig. 2 A shows results obtained using pECK, a probe containing the germine J\(_k\) and C\(_\alpha\) segments (12). Any V\(_k\) to J\(_k\) rearrangement generating restriction fragments of a different size than the fusion partner can be detected using this approach. Hybrids that give bands of different mobilities are likely to be independently derived (4).
Figure 2. Southern blot hybridization of hybridomas using V_{k12A}. Genomic DNA was isolated from hybridomas expressing V_{k12A}, cut with BamHI, and analyzed by Southern blotting to pECK (A) or pKP6 (B). The following hybrids were represented: 2352-41 (lanes 1), 2352-37 (lanes 2), 2352-30 (lanes 3), 2352-12 (lanes 4), 2352-33 (lanes 5), 2352-23 (lanes 6), 2352-57 (lanes 7), and 2352-9 (lanes 8). (A, →) Location of the fragments containing the V_{k12A} rearrangement. Sizes of fragments from different hybrids correlate with the different J_{k} segment use. (↑) Bands that are contributed by the fusion partner SP2/0. (*) Other potential rearrangements. Only the fragments in lanes 3 and 8 were used as evidence of independent origin (see Table 2). The other two had intensities that were indicative of submolar concentrations. Molecular weight marker positions were indicated on the margins.

Despite the complex hybridization pattern of the fusion partner, SP2/0, it was possible to detect V_{k} rearrangements by hybridization to pECK (Table 2). For example, in the set of V_{k12A} rearrangements shown in Fig. 2 A, the presumed V_{k12A} containing band is between 17 and 18.5 kb. The differences in this range of sizes were found to correlate with the distinct J_{k} use in these hybrids (Table 2). Some hybrids rearranged both V_{k} alleles (e.g., 2352-30 in lane 3, and 2352-9 in lane 8 of Fig. 2 A). Analogous experiments were performed using pJ11 (11), a probe extending across the J_{k} segments (Table 2).

Hybridization to pKP6, a fragment located immediately upstream of J_{k1} (13), detects reciprocal products of inversionsal rearrangements whose size is different from the germ-line SP2/0 band. In the case of the V_{k12A} set of hybrids shown (Fig. 2 B), the two rearrangements to J_{k5}, 2352-12 (lane 4) and 2352-57 (lane 7), share a band of 9.3 kb. However, the reciprocal products in hybrids that showed V_{k12A} rearrangements to other J_{k} segments provided additional evidence for their independence. By using probes for the J_{k} loci as well as pKP6, sufficient data were obtained to distinguish each of the hybrids (Table 2). Hence, the independent origin of hybridomas in this study was established by differences in their J_{k} use, genomic DNA hybridization results, and V_{k}-J_{k} sequences.

Estimate of the Total V_{k} Repertoire. Based on the observed

Figure 3. Diagram of two alternative rearrangement pathways that could lead to a V_{k12A}/J_{k5} L chain. Two successive recombination events on the same chromosome are necessary to explain the fact that a productive V_{k12A}/J_{k5} rearrangement is not linked with its reciprocal joint. The diagram on the left (Pathway A) depicts the completion of the V_{k12A}/J_{k5} rearrangement before the rearrangement of gene X to an orphaned J_{k}. Retention of the rp demands that both rearrangements occur by inversion. However, approximately half of the secondary rearrangements should result in deletion of sequences upstream of J_{k1} (e.g., genes pointing away from C_{k} should have an equal chance of rearranging as genes pointing toward C_{k}, according to data in reference 21). Hybridization data obtained using pKP6 did not support pathway A. Pathway B (right) assumes that gene X rearranges first by inversion to any other J_{k} except J_{k5}. In this case, the rearrangement of V_{k12A} to J_{k5} in a second recombination event would retain the rp regardless of whether V_{k12A} or gene X (shown here) are C_{k} proximal. Data in Table 2 are more consistent with pathway B.
Table 2. Southern Blot Analysis of the H and L Chain Rearrangement Status in Hybridomas that Share \( V_\epsilon \) and \( J_\epsilon \) Elements

| Hybrid | \( V_\epsilon \) | \( J_\epsilon \) | pECK | pKP6 | pJ11 |
|--------|----------------|----------------|------|------|------|
| 104-10 | 1              | 4              | 9    | ND*  | gl, tg |
| 104-76 | 1              | 4              | ND   | ND   | ND   |
| 104-85 | 4              | 5              | ND   | ND   | -    |
| 104-12 | 4              | 5              | 6.4  | 14.2 | -    |
| 104-41 | 4              | 5              | 6.4  | 14.2 | gl, tg |
| 104-68 | 4              | 5              | 6.4, 8.4 | 15 | -    |
| 104-89 | 4              | 5              | 6.4, 8.6 | 14.2, 13.5 | -    |
| 104-92 | 4              | 5              | 6.4  |    | -    |
| 2352-12 | 12             | 1              | 18.5 | 9.3, 14.2 | -    |
| 2352-57 | 12             | 1              | 18.5 | 9.3  | -    |
| 2352-23 | 12             | 2              | 18   | 14.2, 19 | -    |
| 2352-33 | 12             | 2              | 18   | 10.5 | -    |
| 104-9   | 12             | 2              | ND   | ND   | gl, tg |
| 104-71 | 12             | 2              | 18   | 10.5 | ND   |
| 104-14 | 12             | 5              | 17   | 9.3  | -    |
| 104-30 | 12             | 5              | 17, 9 | 8.0 | -    |
| 104-116 | 12            | 5              | 17   | -    | ND   |
| 2352-9 | 12             | 5              | 17, 8.1 | 14.2 | -    |
| 2352-25 | 12             | 5              | ND   | ND   | -    |
| 2352-30 | 12             | 5              | 17, 4.0 | - | -    |
| 2352-34 | 12             | 5              | ND   | ND   | -    |
| 2352-37 | 12             | 5              | 17   | 10.5 | gl, tg |
| 2352-38 | 12             | 5              | ND   | ND   | -    |
| 2352-41 | 12             | 5              | 17   | 9.3  | -    |
| 104-74 | 19             | 1              | ND   | ND   | -    |
| 104-78 | 19             | 1              | 11.0 | ND   | gl, tg |

Hybridomas with identical \( V_\epsilon,J_\epsilon \) gene segment use from the 104 and 2352 mouse fusions are listed. The table identifies the \( V_\epsilon \) and \( J_\epsilon \) segments used and lists the sizes of restriction fragments that hybridized to pECK, pKP6, or pJ11 (for details see Materials and Methods). Most hybrids could be distinguished by at least one of the probes, whereas certain other hybrids were distinguished by their V-J junction sequences (Radic, Marko Z., manuscript in preparation).

* No hybrids had the SJL pKP6 gl band, suggesting that both mice were heterozygous (B16/BALB/c) upstream of J.

\( V_\epsilon \) gene use and the independent nature of each hybrid, one can estimate the total pool of endogenous L chains that are likely to be associated with the \( V_\epsilon 3H9 \) H chain in vivo. Using the number of single, double and triple occurrences of the observed \( V_\epsilon \) genes, we calculated the maximum likelihood estimate (MLE) for different L chains in the repertoire as a whole. The \( V_\epsilon 5B \) and \( V_\epsilon 12A \) genes that were isolated 7 and 16 times, respectively, were not used in deriving the MLE, because they may be favored by the editing mechanism (see above) and therefore may not be appropriate for estimating the overall \( V_\epsilon \) repertoire. The MLE for the L chain repertoire in \( V_\epsilon 3H9 \) mice was 35, with two-sided 90% confidence bounds of 21 and 85. Since the two most frequent \( V_\epsilon \) genes were not used in this conservative estimate, we subsequently added them to the total, thus raising the estimate of the available repertoire to 37 genes.

Analysis of Reciprocal Products. In theory, functional rearrangements to any \( J_\epsilon \) segment can be the initial event that yields a functional L chain. Yet, in practice, many \( V_\epsilon \) loci show evidence of multiple rearrangements. For example, most of the \( J_\epsilon 5 \) rearrangements in the antihemagglutinin Sb response are preceded by a previous rearrangement on the same allele (18). As our sample was also characterized by a high frequency of \( J_\epsilon 5 \), we examined the likelihood of secondary rearrangements. We focused our analysis on \( V_\epsilon 12A \) rearrangements (Fig. 3). This group of rearrangements contained examples in which the same \( V_\epsilon \) gene was joined to different \( J_\epsilon \) segments. Rearrangement, we subsequently added them to the total, thus raising the estimate of the available repertoire to 37 genes.
rangements to J1 must be the results of primary events. Therefore, they allowed us to determine whether V1,12A rearrangements by a deletion or an inversion. Since both hybridomas, 2352-2 (Fig. 2 B, lane 4) and 2352-57 (Fig. 2 B, lane 7), which had a V1,12A/J1 rearrangement also shared a 9.3-kb pKP6 band, it is highly likely that V1,12A rearranges by inversion. However, the size of pKP6 containing fragments from other V1,12A hybrids is incompatible with the idea that these are reciprocal products (rp) of the functional rearrangement (Table 2).

At least two alternative pathways exist which could explain the observed results (Fig. 3). In pathway A, the V1,12A-J1,5 joint is formed first, leaving upstream J5s free to further rearrange. Further rearrangements of inverted J5s have recently been shown to occur (19). In pathway B, an unknown Vx rearranges first to any J, except J,5. The retention of the rp in the first rearrangement is expected, according to the results of Harada and Yamagishi (20) which indicate that the majority of primary rearrangement events are inversions. In this scheme (Fig. 3), the V1,12A rearranges second. The pKP6 hybridization data (Table 2) show that eight of ten V1,12A rearrangements to downstream J5s retained at least one pKP6 fragment. These results support pathway B because the secondary rearrangement of V1,12A should retain the first rp regardless of the relative positions of V1,12A and the Vx gene that participated in the first rearrangement. Our data did not support pathway A which, since about half of all Vx genes face away from the Jx locus (21), should result in approximately equal numbers of deletions and inversions. It is therefore probable that rearrangements involving V1,12A and downstream J5s were preceded by other rearrangements.

Discussion

Optimum combinatory (H x L) diversity of antibodies requires that the use of L chains should not be biased by the identity of the H chains. This rule should also apply in the presence of a transgenic H chain. Nevertheless, we found that only a minority of possible L chains was represented in splenic B cell hybridomas from anti-DNA IgM H chain transgenic mice. We sequenced these L chains in order to reveal the cause and the mechanism of the repertoire restriction. Our analysis showed that only 18 Vx genes could account for all 44 L chain transcripts from Vx,3H9 mice. This limited L chain repertoire was expressed by hybrids that produced anti-ssDNA antibodies and those with no DNA specificity (Table 1). The biased representation of L chains led to the absence of entire Vx groups from our sample, and to the disproportionate use of other Vx groups. For example, the group bias that exists in Vx,3H9 mice was most evident among V1,12/13 genes. This Vx group is estimated to contain between two and eight genes (22), or <5% of the total available repertoire, yet it gave rise to nearly 40% of the expressed L chains (Table 1). A further consequence of L chain bias was that certain Vx genes dominated their respective groups (Table 1). In our survey, V1,12A was found in 16 of 17 hybrids expressing genes of the Vx,12 group. We could extrapolate from these data to predict that the available Vx repertoire of the Vx,3H9 mouse is 37 genes, far less than the 200 Vx genes available to normal mice (23).

Although group representation in nontransgenic mice reflects each group's complement of Vx genes (24, 25), the use of Vx genes within a group is not uniform, perhaps reflecting their differential capacity to interact with antigens. This was recently demonstrated by Milstein et al. (26) who analyzed the use of 14 members of the Vx,4/5 group. The authors found a ten to one preference for certain members of this Vx group over others. It is not possible to directly compare the biased representation of the Vx,4/5 group by Vx,5B seen here (Table 1) with the results of Milstein et al. (26), since their approach did not score for Vx,5B. Nevertheless, it appears that the intra-Vx,4/5 bias in Vx,3H9 mice may be even more extreme.

Additional examples of Vx (27, 28) and Vx (28-30) repertoire bias have been observed. These occur early in life (27, 29) or in association with the Ly-1 B cell lineage (28, 30). In addition Gu et al. (30) demonstrated that in adult mice the Vx repertoire is more restricted in mature B cells than in pre-B cells. A substantially biased repertoire of Vx and Vx1 characterizes the autoimmune strain MRL/lpr. In mice with signs of progressed disease, the J558 H chain family accounts for the majority of Ig transcripts in the spleen, whereas different Vx groups may be overrepresented in different mice (31). It is unlikely that the Vx gene bias seen here parallels any of the studies above since none of the Vx genes shown in Table 1 matched genes whose overrepresentation was previously reported (27, 28).

Jx segment use in our L chain survey (Table 1 and Fig. 1) also differs from the Jx use in B cells of normal, adult mice. Whereas normal use is biased toward J1,1 and J1,2 (15, 16), nearly half of the 44 rearrangements from Vx,3H9 mice were to J5. The high frequency of J5 along with the Vx repeats in turn leads to the recurrence of Vx,Jx pairs. Since the use of identical gene segments raises the possibility that these hybridomas are from one or a few expanded B cell clones (4), we compared their silent H and L chain rearrangements. There was no evidence for clonal relatedness among the hybrids, including those with V1,12A-J1,5 rearrangements. Clearly, other reasons must account for the Jx segment bias.

One reason for the restricted Vx and Jx repertoire may be correction of rearranged V genes. Two kinds of corrective mechanisms are known. In Vx replacement a V-D-J can become a substrate for further rearrangements because of a sequence, embedded in most Vx genes, that is identical to the conserved heptamer signal which is required by the V-D-J recombinase (32). Nonfunctional L chain rearrangements can be corrected using a different mechanism. As mentioned above, most V-J rearrangements involve Jx,1 or Jx,2, thus leaving the downstream Jx segments intact and available for further Vx rearrangements. Correction by secondary rearrangements can occur as shown by the linkage of two Vx-Jx rearrangements on the same chromosome (13). Both editing mechanisms reduce the repertoire. If the primary rearrangement is deletional, then the pool of editing donors will be smaller. Furthermore, the Jx repertoire will be reduced, since the number of Jx
available for editing will be less than the four Jα segments available to the primary rearrangement. Further editing may delete the Vα locus entirely, and lead to the expression of Vκ L chains (33).

The same mechanism that allows the correction of non-functional Vκ-Jκ joints may also operate when selective pressure is applied against the surface receptor containing the Vκ-Jκ product. Immunotherapy with an antiidiotype toxin conjugate was applied against a murine B cell lymphoma and led to variants that had replaced the productive Vκ-Jκ joint with secondary rearrangements to downstream Jκ segments (34).

This way of changing the idiotype of a B cell receptor suggests a hypothesis for explaining the L chain bias in Vκ3H9 mice. We propose that tolerance to dsDNA (and presumably certain other autoantigens) can cause autoreactive B cells to edit their L chains. Consistent with this hypothesis is that L chains which remain in the Vκ3H9 repertoire prevent dsDNA binding. In the case of the Vκ3H9 transgene, the hypothesis predicts a limited repertoire of Vκ genes, since many L chains of the mouse bind dsDNA when combined with Vκ3H9 (4-6). Although simple deletion of self-reactive B cells from an initially random population could lead to a similar Vκ bias, the concomitant, high frequency of Jκ5 is more consistent with an editing mechanism that activates secondary rearrangements.

Negative selection may even be capable of editing transgene-encoded Ig receptors. Gay et al. (35) analyzed transgenic mice capable of producing both the H and the L chains of the Vκ3H9 antibody. As neonates, these 3H9 H chain/Vκ4 L chain mice are profoundly B cell-deficient, but as adults they have near normal numbers of B cells. Hybridomas derived from these adult mice did not bind dsDNA even though they expressed both transgenic H chain and L chain. These hybrids also express endogenous L chains which are identical to the L chains described here (Table 1). It is thought that these endogenous L chains preferentially associate with the 3H9 H chain and thereby yield antibody that does not bind dsDNA. Thus, preferential H-L association can edit dsDNA-specific receptors and permit B cells to escape deletion.

Secondary, and indeed multiple, rearrangements of L chain alleles may account for the overexpression of Vκ L chains as seen in other Ig transgenic lines (36-38). As stated above, L chain editing is expected to increase the proportion of Vκ L chains in the repertoire (33). In fact, an elevated proportion of Vκ was interpreted as evidence of L chain editing (37). An increased frequency of Vκ would also be the predicted outcome here, were it not for the fact that this L chain forms anti-dsDNA antibodies in combination with Vκ3H9 (5). Receptor editing in normal, i.e., nontransgenic mice may be a common occurrence. This is supported by the remains of edited Vκ genes that show no obvious defects (20). Therefore, functional genes may be edited in analogous ways as aberrant Vκ-Jκ joints. A case in point is the in-frame rearrangement of a Vκ8 gene to Jκ1 found as remnant in the PC3609 plasmacytoma (13). A possible reason for the replacement of this functional Vκ gene may be the rare arginine codon that was generated during Vκ-Jκ joining (13). Arginines are frequently involved in DNA binding and they play an important role in anti-DNA antibodies (8). Indeed, Tillman et al. (6) found arginines at the Vκ8-Jκ1 junction of anti-DNA antibodies. This example suggests that L chain editing as described here is part of a normal cellular mechanism that regulates autoreactive Ig receptors.

Editing may also occur during TCR assembly. Studies have shown that functional Vκ-Jκ joints can be replaced by additional rearrangements (39). In fact, recent evidence suggests that in the absence of a positive growth signal, T cells may fail to shut down recombination, possibly reflecting the increased frequency of secondary rearrangement events (40). It is intriguing to note that a shift to downstream Jκ segments may also be associated with ontogenic progression (41).

Editing may be invoked for other reasons besides aberrant rearrangement and self-reactivity. For example, some L chains may form unstable H-L pairs with Vκ3H9. Poor H-L pairing has been suggested to explain that biased repertoire of endogenous L chains in a H chain-only transgenic (42), as well as the replacement of functional Vκ-Jκ rearrangements by secondary events (20). Inefficient pairing could also account for the L chain bias in other Ig transgenics which show a restricted use of endogenous L chains (36-38). Nevertheless, unstable H-L pairing does not fully explain the Jκ bias in Vκ3H9 mice, since, based on in vivo and in vitro data (4-6), we expect Vκ3H9 to be competent for pairing with a wide range of L chains.

In conclusion, we interpret the restricted Vκ gene use in Vκ3H9 transgenic mice as evidence for an editing mechanism that excludes a large fraction of endogenous L chains from the repertoire. In our view, editing must be the outcome of the surface expression of Ig receptors whose affinity for dsDNA is dictated by many of the potential Vκ3H9-Vκ pairs. Since editing is proposed to occur via secondary L chain rearrangements, it follows that the regulation of anti-dsDNA specificity allows sufficient time for the rescue of B cells by the replacement of dsDNA binding L chains with L chains that do not.

The authors thank Ms. Anita Cywinski for technical assistance and insights into cDNA extension and sequencing procedures; Ms. Joni Brill-Dashoff for skilled assistance with DNA hybridizations; and Ms. Violet Hay for assistance with cell culture and animal husbandry. Thanks also go to Dr. Richard R. Hardy for FACS® time. The secretarial assistance of Ms. Christine Hamilton is appreciated.
Support for this work was provided to M. Z. Radic by the Julius Erving Lupus Research Fund and an allocation made to the Dean of the Medical College of Pennsylvania by the Allegheny Singer Research Institute; to J. Erikson by the National Institutes of Health (NIH AI-32137); to S. Litwin by a Fox Chase Cancer Center Core Grant; and to M. Weigert by the NIH (GM-2094, CA-06927, and RR-05539), an appropriation from the Commonwealth of Pennsylvania, and the Sheryl N. Hirsch Award from the Lupus Foundation of Philadelphia.

Address correspondence to Dr. Martin Weigert, Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111.

Received for publication 28 September 1992 and in revised form 14 January 1993.

References

1. Goodnow, C.C., J. Crosbie, S. Adelstein, T.B. Lavoie, S.J. Smith-Gill, R.H. Brink, H. Pritchard-Briscoe, J.S. Wotherspoon, R.H. Loblay, K. Raphael, R.J. Trent, and A. Basten. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature (Lond.) 334:676.

2. Nemazee, D.A., and K. Burki. 1989. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. Nature (Lond.) 337:562.

3. Goodnow, C.C., J. Crosbie, S. Adelstein, T.B. Lavoie, S.J. Smith-Gill, R.H. Brink, H. Pritchard-Briscoe, J.S. Wotherspoon, R.H. Loblay, K. Raphael, R.J. Trent, and A. Basten. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature (Lond.) 334:676.

4. Nemazee, D.A., and K. Burki. 1989. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. Nature (Lond.) 337:562.

5. Erikson, J., M.Z. Radic, S.A. Camper, R.R. Hardy, C. Carmack, and M. Weigert. 1991. Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice. Nature (Lond.) 349:331.

6. Badley, J.E., G.A. Bishop, T. St. John, and J.A. Frelinger. 1989. Structural patterns in anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. J. Exp. Med. 171:265.

7. Badley, J.E., G.A. Bishop, T. St. John, and J.A. Frelinger. 1989. Structural patterns in anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. J. Exp. Med. 171:265.

8. Radic, M.Z., M.A. Mascelli, J. Erikson, H. Shan, and M. Weigert. 1991. Ig H and L chain contributions to autoimmune mice arise by clonal expansion and somatic mutation. J. Exp. Med. 171:265.

9. Shlomchik, M., M. MasceUi, A. Marshak-Rothstein, and M. Weigert. 1990. Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. J. Exp. Med. 171:265.

10. Radic, M.Z., M.A. Mascelli, J. Erikson, H. Shan, and M. Weigert. 1991 Ig H and L chain contributions to autoimmune specificities. J. Immunol. 146:176.

11. Strohal, R., A. Helmberg, G. Kroemer, and R. Kofler. 1989. Expression of murine antibody genes in transgenic mice. Nature (Lond.) 334:676.

12. Gill, R.A. Brink, H. Pritchard-Briscoe, J.S. Wotherspoon, R.H. Loblay, K. Raphael, R.J. Trent, and A. Basten. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature (Lond.) 334:676.

13. Feddersen, R.M., and B.G. VanNess. 1985. Double recombination of a single immunoglobulin κ-chain allele: implications for the mechanism of rearrangement. Proc. Natl. Acad. Sci. USA. 82:4793.

14. Yui, K., A. Bhandoola, M.Z. Radic, S. Komori, M. Katsumata, and M.I. Greene. 1992. Inhibition of abnormal T cell development and autoimmunity in gld mice by transgenic T cell receptor β chain. Eur. J. Immunol. 22:1693.

15. Wood, D.L., and C. Coleclough. 1984. Different joining region J elements of the murine κ immunoglobulin light chain locus are used at markedly different frequencies. Proc. Natl. Acad. Sci. USA. 81:4756.

16. Nishi, M., T. Kataoka, and T. Honjo. 1985. Preferential rearrangement of the immunoglobulin κ chain joining region J1 and J2 segments in mouse spleen DNA. Proc. Natl. Acad. Sci. USA. 82:6399.

17. Mehta, C.R., and M.Z. Radic. 1983. A network algorithm for performing Fisher's exact test in vxc contingency tables. Journal American Statistics Assoc. 78:427.

18. Clarke, S., and S. McCray. 1991. A shared κ reciprocal fragment and a high frequency of secondary Jκ rearrangements among influenza hemagglutinin specific B cell hybridomas. J. Immunol. 146:343.

19. Huber, C., H.-G. Klobeck, and H.G. Zachau. 1992. Ongoing Vκ-Jκ recombination after formation of a productive Vκ-Jκ coding joint. Eur. J. Immunol. 22:1561.

20. Harada, K., and H. Yamagishi. 1991. Lack of feedback inhibition of Vκ gene rearrangement by productively rearranged alleles. J. Exp. Med. 173:409.

21. Shapiro, M.A., and M. Weigert. 1987. How immunoglobulin Vκ genes rearrange. J. Immunol. 139:3834.

22. Strohal, R., A. Helmberg, G. Kroemer, and R. Kofler. 1989. Mouse Vκ gene classification by nucleic acid sequence similarity. Immunogenetics. 30:475.

23. Honjo, T. 1985. Immunoglobulin light chain genes. Annu. Rev. Immunol. 3:1-499.

24. Kalled, S.L., and P.H. Brodeur. 1991. Utilization of Vκ families and Vκ exons: implications for the available B cell repertoire. J. Immunol. 147:3194.

25. Teale, J.M., and E.G. Morris. 1989. Comparison of Vκ gene family expression in adult and fetal B cells. J. Immunol. 143:2768.

26. Milstein, C., J. Even, J.M. Jarvis, A. Gonzalez-Fernandez, and E. Gherardi. 1992. Non-random features of the repertoire expressed by the members of one Vκ gene family and of the V-J recombination. Eur. J. Immunol. 22:1627.

27. Lawler, A.M., J.F. Kearney, M. Kuehl, and P.J. Gearhart. 1989. Early rearrangements of genes encoding murine immunoglobul-
ulin κ chains, unlike genes encoding heavy chains, use variable gene segments dispersed throughout the locus. Proc. Natl. Acad. Sci. USA. 86:6744.

28. Hardy, R.R., C.E. Carmack, S.A. Shinton, R.J. Riblet, and K. Hayakawa. 1989. A single Vκ gene is utilized predominantly in anti-BrMRBC hybridomas derived from purified Ly-1 B cells. 1989. J. Immunol. 142:3643.

29. Yancopoulos, G.D., S.V. Desiderio, M. Paskind, J.F. Kearney, D. Baltimore, and F.W. Alt. 1984. Preferential utilization of the most Jκ-proximal Vκ gene segments in pre-B cell lines. Nature (Lond.). 292:629.

30. Gu, H., D. Tarlinton, W. Müller, K. Rajewsky, and I. Förster. 1991. Most peripheral B cells in mice are ligand selected. J. Exp. Med. 173:1357.

31. Teale, J.M., K.M. Sievers, R.R. Crawley, and B.L. Kotzin. 1992. Ig Vκ family repertoire of plasma cells derived from autoimmune MRL mice. J. Immunol. 148:142.

32. Kleinfield, R., R.R. Hardy, D. Tarlinton, J. Dangl, L.A. Herzenberg, and M. Weigert. 1986. Recombination between an expressed immunoglobulin heavy-chain gene and a germline variable gene segment in a Ly1 B cell lymphoma. Nature (Lond.). 322:843.

33. Durdk, J., M.W. Moore, and E. Selsing. 1984. Novel κ light-chain gene rearrangements in mouse λ chain-producing B lymphocytes. Nature (Lond.). 307:749.

34. Levy, S., M.J. Campbell, and R. Levy. 1989. Functional immunoglobulin light chain genes are replaced by ongoing rearrangements of germline Vκ genes to downstream Jκ segments in a murine B cell line. J. Exp. Med. 170:1.

35. Gay, D., T. Saunders, S. Camper, and M. Weigert. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. J. Exp Med. In press.

36. Rath, S., A. Nisonoff, E. Selsing, and J.M. Durdk. 1991. B cell abnormalities induced by a μ Ig transgene extend to L chain isotype usage. J. Immunol. 146:2841.

37. Nemazee, D., D. Russell, B. Arnold, G. Haemmerling, J. Allison, J.F.A.P. Miller, G. Morahan, and K. Buerki. 1991. Clonal deletion of autospecific B lymphocytes. Immunol. Rev. 122:117.

38. Jacomini, J., N. Yannoutsis, S. Bandyopadhay, and T. Imanishi-Kari. 1991. Endogenous immunoglobulin expression in mu transgenic mice. Int. Immunol. 3:185.

39. Marolleau, J.-P., J.D. Fondell, M. Malissen, J. Trucy, E. Barbier, K.B. Marcu, P.-A. Cazenave, and D. Prim. 1988. The joining of germ-line Vα to Jκ genes replaces the preexisting Vα-Jκ complexes in a T cell receptor α,β positive T cell line. Cell. 55:291.

40. Borgulya, P., H. Kishi, Y. Uematsu, and H. von Boehmer. 1992. Exclusion and inclusion of α and β T cell receptor alleles. Cell. 69:529.

41. Thompson, S.D., M. Larche, A.R. Manzo, and J.L. Hurwitz. 1992. Diversity of T cell receptor α and β isotypes in normal and pathological lymphoid tissues. Cell. 69:95.

42. Brombacher, F., G. Kohler, and H. Eibl. 1991. B cell tolerance in mice transgenic for anti-CD8 immunoglobulin μ chain. J. Exp. Med. 174:1335.

43. Goshorn, S.C., E. Retzel, and R. Jemmerson. 1991. Common structural features among monoclonal antibodies binding the same antigenic region of cytochrome c. J. Biol. Chem. 266:2134.