B Lymphocytes May Escape Tolerance by Revising Their Antigen Receptors

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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_κ and J_κ 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_κ group, were used more than once and accounted for three fourths of all sequences. Based on the distribution of V_κ genes, the L chain repertoire in this line of transgenic mice was estimated at 37 V_κ genes. The most frequently observed gene, a member of the V_κ 12/13 group, was identified in 16 hybrids. In addition, the majority of V_κ genes used $J_\kappa 5$. We interpret the skewed representation of V_κ and J_κ gene segments to result from negative selection. Based on the data, we suggest that V_κ 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_H3H9 and $V_\kappa 8$ 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_H3H9 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_H3H9 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_κ and J_κ 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, $J_\kappa 5$ was overutilized. The data suggested that the major driving force shaping the L chain use in V_H3H9 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_H3H9 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_H3H9 mouse (No. 2352) which were sorted for the presence of the B220 and V_κ 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.

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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)$^+$ RNA 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_κ segments. Since the SP2/0 fusion partner contains sterile transcripts from an out-of-frame V_κ21E-J_κ2 rearrangement, J_κ2 oligo (5′-CCCTCCGAACGTGTAAGG-3′) extensions from each hybrid were compared with the products from SP2/0. Authentic V_κ-J_κ2 extension products could readily be detected using that approach.

The J_κ4 (5′-TATTTCCAACTTTGTCCCCGAG-3′) and J_κ5 (5′-GAGCTCCAGCTTGGTCCCAGCAC-3′) oligos that were used did not crosshybridize with other J_κ segments or with each other, thus allowing unambiguous assignments. In contrast, it was found that the J_κ1 oligo (5′-TGATTTCCAGCTTCCTGCCTCCAC-3′) was capable of extending mRNA starting from J_κ1 and J_κ2 sequences. Therefore, unambiguous J_κ1 or J_κ2 assignments were made after sequencing cDNAs obtained by reverse transcription from an end-labeled C_κ-specific oligo and gel purification of full size extension products from denaturing PAGE (4).

Sequence Analysis. All rearrangements to J_κ1 or J_κ2 were sequenced by chemical degradation of cDNAs obtained by reverse transcription with a C_κ-specific oligo, as described (8). Rearrangements to J_κ4 or J_κ5 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 J_H segments and including the H chain enhancer (11). L chain rearrangements were analyzed by hybridization to pECK (12), a fragment containing all four functional J_κ segments, as well as pKP6 (13), a 0.9-kb EcoRI fragment directly upstream of J_κ1.

DNA Binding Assays. All IgM antibodies were concentrated by ammonium sulfate precipitation, followed by resuspension and dialysis against PBS. Antibody concentrations were determined after adsorption to microtiter wells coated with goat anti-mouse IgM (Fisher Scientific, Pittsburgh, PA) and blocked with 1% BSA in PBS. Unbound material was washed off, and bound IgM was quantitated following a 1-h incubation with alkaline phosphatase–labeled goat anti-mouse serum Ig (Fisher Scientific), washing, and reaction with *p*-nitrophenol. Absorbance was quantitated by spectrophotometry in a microplate reader (Bio-Rad Laboratories) set at 405 nm. Binding to ss and dsDNA was measured as described (14). Salmon sperm DNA was treated with S1 nuclease (United States Biochemical Corp., Cleveland, OH) for 5 min at 37°C, extracted with phenol, and precipitated by addition of 96% ethanol to obtain a stock of dsDNA. Photobiotinylation and solution phase equilibrium binding to ss and dsDNA were performed as described (5). Solutions containing antibody–DNA complexes and/or unbound reagents were transferred to microtiter plates that were coated with avidin D (Vector Laboratories, Inc., Burlingame, CA) and blocked with 1% BSA in PBS. After adsorption for 60 min, plates were washed three times, and the retained IgM were quantitated using an alkaline phosphatase–conjugated goat anti-mouse IgM reagent (Fisher Scientific), as described above.

Results

Gene Segment Use in Hybridomas. To correlate Ig V region expression in splenic B cells from V_H3H9 mice with their binding properties, hybridomas from two mice of the transgenic line were generated. Two types of hybridomas were recovered: those whose antibodies bound ssDNA and those whose antibodies had no detectable binding to DNA (Table 1). It was conceivable that the loss of DNA binding capacity observed in these hybridomas was due to inactivation of the H chain transgene 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 Cμ oligonucleotide. None of the hybrids analyzed expressed an endogenous IgM mRNA. Instead, only the V_H3H9-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_κ 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_H3H9 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_κ genes. Only nine of the more than twenty V_κ groups were observed. Moreover, the representation of V_κ groups that were observed was not proportional to their size. In addition to the disproportionate representation of certain V_κ groups, recurrent V_κ genes also characterized this sample. Although 12 of the V_κ 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_κ genes is also unusual in that J_κ5 accounted for the majority (43%) of the rearrangements observed (Table 1). This is different from J_κ gene segment frequencies in splenic B cells of nontransgenic mice. Various investigators have estimated that between 35 and 47% of V_κ genes rearrange to J_κ1, whereas only 6–25% use J_κ5 (15, 16). We compared the frequencies of all four J_κ 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_κ usage in LPS-treated splenic B cells of BALB/c mice described above ($p \leq 0.008$).

Most of the J_κ5 segments (17 of 19) were associated with V_κ genes that were isolated more than once in our survey (Fig. 1), whereas J_κ use in single V_κ isolates favored J_κ1 (9

Table 1. *Summary of V_κ and J_κ Segments Used in 42 Hybridomas from V_H3H9 Mice*

V_κ Group	V_κ Gene	J_κ1	J_κ2	J_κ4	J_κ5	Total	ssDNA	MLE
1 (4–6)	1A	1	–	–	–	1	–	20 (18–25)
	1B	–	1	2	–	3	–	
4/5 (25–50)	5A	1	–	–	–	1	–	
	5B	–	1	–	6	7*	+	37* (23–87)
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_κ gene used. V_κ genes were assigned to a V_κ group and distinguished by a capital letter suffix. In keeping with this nomenclature, a new member of the V_κ21 group is called V_κ21I. Repeats of each V_κ gene, numbers of rearrangements to each of the four J_κ segments, and ssDNA affinities were indicated. Two hybridomas, 2352-37 and 2352-46, expressed two in-frame V_κ rearrangements, thus 42 hybrids yielded 44 L chain sequences. One V_κ gene, 1G1, belonging to an as yet unclassified V_κ 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_κ5B and V_κ12A genes. In the presence of those two most frequently used V_κs, the MLE predicted a repertoire of 20–25 V_κ genes. A more conservative calculation of the MLE is indicated by an asterisk and did not include these two genes. Therefore, those two V_κ 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_H3H9 mice was much less than the potential germline repertoire of ~200 V_κ genes.

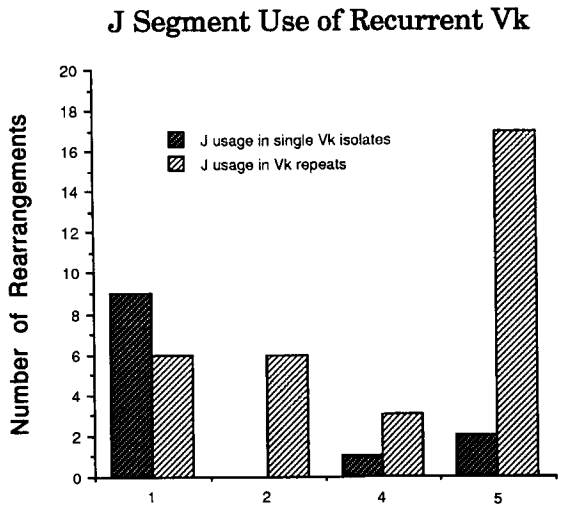

Figure 1. J_κ segments associated with single and recurrent V_κ genes. The number of rearrangements to each of the J_κ segments was plotted for V_κ genes seen only once in this survey (*darker bars*) and V_κ genes that were seen at least twice (*lighter bars*).

of 12 examples). Overall, the J_κ use in single isolates and repeats was significantly different ($p \leq 0.0042$). Thus, the recurrent V_κ genes accounted for the overutilization of J_κ5. Two of the recurrent genes, V_κ12A and V_κ5B, accounted for 16 J_κ5 rearrangements, although both were also seen to associate with other J_κ segments.

Independent Origin of Recurrent V_κ-J_κ Pairs. Several of the hybrids from either fusion expressed L chains composed of the same V_κ and J_κ gene segments. One possible reason for such recurrent V_κ-J_κ 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_κ12A are shown in Fig. 2. Fig. 2 *A* shows results obtained using pECK, a probe containing the germline J_κ and C_κ segments (12). Any V_κ to J_κ 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).

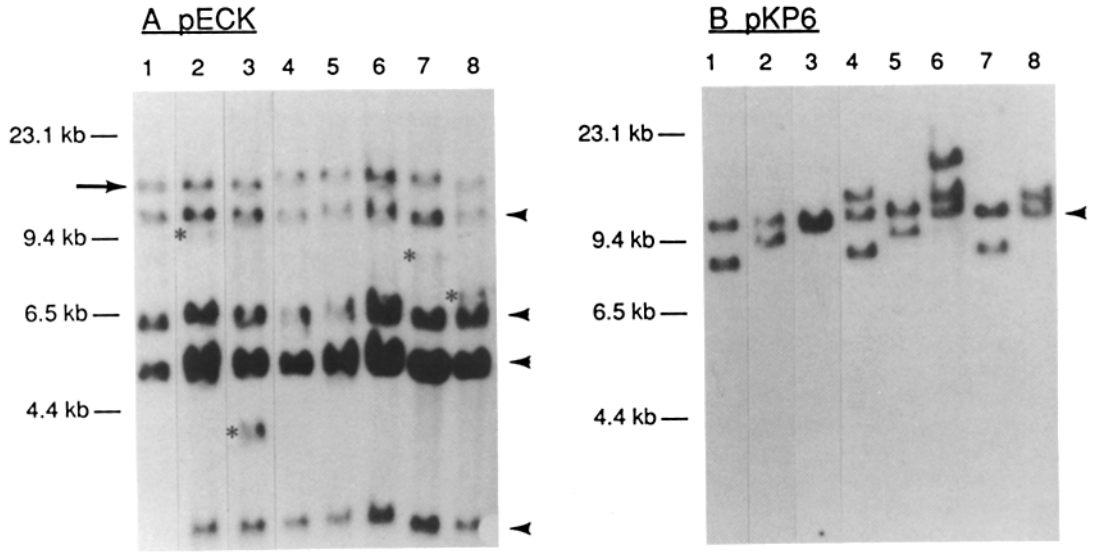

Figure 2. Southern blot hybridization of hybridomas using V_κ12A. Genomic DNA was isolated from hybridomas expressing V_κ12A, cut with BamHI, and analyzed by Southern blotting to pECK (*A*) or pKP6 (*B*). The following hybrids were represented: 2352-41 (lanes *1*), 2352-37 (lanes *2*), 2352-30 (lanes *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_κ12A rearrangement. Sizes of fragments from different hybrids correlate with the different J_κ 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_κ rearrangements by hybridization to pECK (Table 2). For example, in the set of V_κ12A rearrangements shown in Fig. 2 *A*, the presumed V_κ12A containing band is between 17 and 18.5 kb. The differences in this range of sizes were found to correlate with the distinct J_κ use in these hybrids (Table 2). Some hybrids rearranged both V_κ 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_H segments (Table 2).

Hybridization to pKP6, a fragment located immediately upstream of J_κ1 (13), detects reciprocal products of inversional rearrangements whose size is different from the germline SP2/0 band. In the case of the V_κ12A set of hybrids shown (Fig. 2 *B*), the two rearrangements to J_κ1, 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_κ12A rearrangements to other J_κ segments provided additional evidence for their independence. By using probes for the J_H and J_κ 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_κ use, genomic DNA hybridization results, and V_κ-J_κ sequences.

Estimate of the Total V_κ Repertoire. Based on the observed

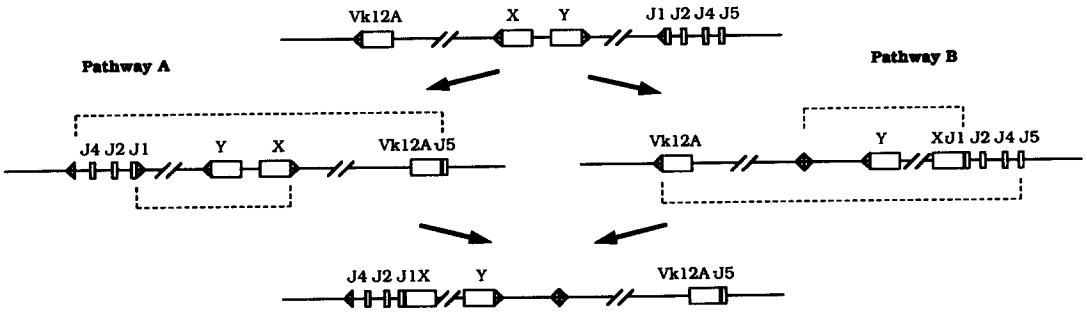

Figure 3. Diagram of two alternative rearrangement pathways that could lead to a V_κ12A/J_κ5 L chain. Two successive recombination events on the same chromosome are necessary to explain the fact that a productive V_κ12A/J_κ5 rearrangement is not linked with its reciprocal joint. The diagram on the left (*Pathway A*) depicts the completion of the V_κ12A/J_κ5 rearrangement before the rearrangement of gene X to an orphaned J_κ. 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_κ1 (e.g., genes pointing away from C_κ should have an equal chance of rearranging as genes pointing toward C_κ, 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_κ except J_κ5. In this case, the rearrangement of V_κ12A to J_κ5 in a second recombination event would retain the rp regardless of whether V_κ12A or gene X (shown here) are C_κ 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_κ and J_κ Elements*

Hybrid	V_κ	J_κ	pECK	pKP6	pJ11
104-10	1	4	9	ND*	gl, tg
104-76	1	4	ND	ND	ND
104-85	4	5	ND	ND	-, tg, 5.6
104-12	4	5	6.4	14.2	-, tg, 5.7, 6.1
104-41	4	5	6.4	14.2	gl, tg, 9.0
104-68	4	5	6.4, 8.4	15	-, tg, 5.6
104-89	4	5	6.4, 8.6	14.2, 13.5	-, tg, 6.2
104-92	4	5	6.4	7.5	-, tg, 6.1
2352-12	12	1	18.5	9.3, 14.2	-, tg
2352-57	12	1	18.5	9.3	-, tg, 5.7
2352-23	12	2	18	14.2, 19	-, tg
2352-33	12	2	18	10.5	-, tg 5.6
104-9	12	2	ND	ND	gl, tg
104-71	12	2	18	10.5	ND
104-14	12	5	17	9.3	-, tg, 5.6, 6.1
104-30	12	5	17, 9	8.0	-, tg
104-116	12	5	17	–	ND
2352-9	12	5	17, 8.1	14.2	-, tg
2352-25	12	5	ND	ND	-, tg, 4.2
2352-30	12	5	17, 4.0	–	-, tg, 5.7
2352-34	12	5	ND	ND	-, tg, 5.6
2352-37	12	5	17	10.5	gl, tg, 13
2352-38	12	5	ND	ND	-, tg, 4.1, 4.3
2352-41	12	5	17	9.3	-, tg, 4.3, 5.7
104-74	19	1	ND	ND	-, tg
104-78	19	1	11.0	ND	gl, tg

Hybridomas with identical V_κ-J_κ gene segment use from the 104 and 2352 mouse fusions are listed. The table identifies the V_κ and J_κ 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_κ 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_H3H9 H chain in vivo. Using the number of single, double and triple occurrences of the observed V_κ genes, we calculated the maximum likelihood estimate (MLE) for different L chains in the repertoire as a whole. The V_κ5B and V_κ12A 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_κ repertoire. The MLE for the L chain repertoire in V_H3H9 mice was 35, with two-sided 90% confidence bounds of 21 and 85. Since the two most frequent V_κ 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_κ segment can be the initial event that yields a functional L chain. Yet, in practice, many V_κ loci show evidence of multiple rearrangements. For example, most of the J_κ5 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_κ5, we examined the likelihood of secondary rearrangements.

We focused our analysis on V_κ12A rearrangements (Fig. 3). This group of rearrangements contained examples in which the same V_κ gene was joined to different J_κ segments. Rear-

rangements to $J_\kappa 1$ must be the results of primary events. Therefore, they allowed us to determine whether $V_\kappa 12A$ rearranges 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 $V_\kappa 12A/J_\kappa 1$ rearrangement also shared a 9.3-kb pKP6 band, it is highly likely that $V_\kappa 12A$ rearranges by inversion. However, the size of pKP6 containing fragments from other $V_\kappa 12A$ 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 $V_\kappa 12A$-$J_\kappa 5$ joint is formed first, leaving upstream J_κs free to further rearrange. Further rearrangements of inverted J_κs have recently been shown to occur (19). In pathway B, an unknown V_κ rearranges first to any J_κ except $J_\kappa 5$. 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 $V_\kappa 12A$ rearranges second. The pKP6 hybridization data (Table 2) show that eight of ten $V_\kappa 12A$ rearrangements to downstream J_κs retained at least one pKP6 fragment. These results support pathway B because the secondary rearrangement of $V_\kappa 12A$ should retain the first rp regardless of the relative positions of $V_\kappa 12A$ and the V_κ gene that participated in the first rearrangement. Our data did not support pathway A which, since about half of all V_κ genes face away from the J_κ locus (21), should result in approximately equal numbers of deletions and inversions. It is therefore probable that rearrangements involving $V_\kappa 12A$ and downstream J_κs were preceded by other rearrangements.

Discussion

Optimum combinatory (H × 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 V_κ genes could account for all 44 L chain transcripts from V_H3H9 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 V_κ groups from our sample, and to the disproportionate use of other V_κ groups. For example, the group bias that exists in V_H3H9 mice was most evident among $V_\kappa 12/13$ genes. This V_κ 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 V_κ genes dominated their respective groups (Table 1). In our survey, $V_\kappa 12A$ was found in 16 of 17 hybrids expressing genes of the $V_\kappa 12$ group. We could extrapolate from these data to predict that the available V_κ repertoire of the V_H3H9 mouse is 37 genes, far less than the 200 V_κ genes available to normal mice (23).

Although group representation in nontransgenic mice reflects each group's complement of V_κ genes (24, 25), the use of V_κ 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 $V_\kappa 4/5$ group. The authors found a ten to one preference for certain members of this V_κ group over others. It is not possible to directly compare the biased representation of the $V_\kappa 4/5$ group by $V_\kappa 5B$ seen here (Table 1) with the results of Milstein et al. (26), since their approach did not score for $V_\kappa 5B$. Nevertheless, it appears that the intra-$V_\kappa 4/5$ bias in V_H3H9 mice may be even more extreme.

Additional examples of V_κ (27, 28) and V_H (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 V_H repertoire is more restricted in mature B cells than in pre-B cells. A substantially biased repertoire of V_H and V_L 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 V_κ groups may be overrepresented in different mice (31). It is unlikely that the V_κ gene bias seen here parallels any of the studies above since none of the V_κ genes shown in Table 1 matched genes whose overrepresentation was previously reported (27, 28).

J_κ segment use in our L chain survey (Table 1 and Fig. 1) also differs from the J_κ use in B cells of normal, adult mice. Whereas normal use is biased toward $J_\kappa 1$ and $J_\kappa 2$ (15, 16), nearly half of the 44 rearrangements from V_H3H9 mice were to $J_\kappa 5$. The high frequency of $J_\kappa 5$ along with the V_κ repeats in turn leads to the recurrence of V_κ-J_κ 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 $V_\kappa 12A$-$J_\kappa 5$ rearrangements. Clearly, other reasons must account for the J_κ segment bias.

One reason for the restricted V_κ and J_κ repertoire may be correction of rearranged V genes. Two kinds of corrective mechanisms are known. In V_H replacement a V-D-J can become a substrate for further rearrangements because of a sequence, embedded in most V_H 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 $J_\kappa 1$ or $J_\kappa 2$, thus leaving the downstream J_κ segments intact and available for further V_κ rearrangements. Correction by secondary rearrangements can occur as shown by the linkage of two V_κ-J_κ 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 J_κ repertoire will be reduced, since the number of J_κs

available for editing will be less than the four J_κ segments available to the primary rearrangement. Further editing may delete the V_κ locus entirely, and lead to the expression of V_λ L chains (33).

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

This way of changing the idiotype of a B cell receptor suggests a hypothesis for explaining the L chain bias in V_H3H9 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 V_H3H9 repertoire prevent dsDNA binding. In the case of the V_H3H9 transgene, the hypothesis predicts a limited repertoire of V_κ genes, since many L chains of the mouse bind dsDNA when combined with V_H3H9 (4–6). Although simple deletion of self-reactive B cells from an initially random population could lead to a similar V_κ bias, the concomitant, high frequency of J_κ5 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 original 3H9 antibody. As neonates, these 3H9 H chain/V_κ4 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 V_λ L chains as seen in other Ig transgenic lines (36–38). As stated above, L chain editing is expected to increase the proportion of V_λ L chains in the repertoire (33). In fact, an elevated proportion of V_λ was interpreted as evidence of L chain editing (37). An increased frequency of V_λ would also be the predicted outcome here, were it not for the fact that this L chain forms anti-dsDNA antibodies in combination with V_H3H9 (5).

Receptor editing in normal, i.e., nontransgenic mice may be a common occurrence. This is supported by the remains of edited V_κ genes that show no obvious defects (20). Therefore, functional genes may be edited in analogous ways as aberrant V_κ-J_κ joints. A case in point is the in-frame rearrangement of a V_κ8 gene to J_κ1 found as remnant in the PC3609 plasmacytoma (13). A possible reason for the replacement of this functional V_κ gene may be the rare arginine codon that was generated during V_κ-J_κ 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 V_κ8-J_κ1 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 V_α-J_α 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 J_α 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 V_H3H9. 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 V_κ-J_κ 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 J_κ bias in V_H3H9 mice, since, based on in vivo and in vitro data (4–6), we expect V_H3H9 to be competent for pairing with a wide range of L chains.

In conclusion, we interpret the restricted V_κ gene use in V_H3H9 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 V_H3H9-V_L 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.

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