

Analysis of a T Cell Receptor Gene as a Target of the Somatic Hypermutation Mechanism

By John Hackett, Jr., Chris Stebbins,* Brian Rogerson,†
Mark M. Davis,§ and Ursula Storb

From the Department of Molecular Genetics and Cell Biology, The University of Chicago; the *Committee on Immunology, The University of Chicago, Chicago, Illinois 60637; the †Trudeau Institute, Saranac Lake, New York 12983; the §Howard Hughes Medical Institute and the Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California 94305-5402

Summary

In an effort to identify *cis*-acting elements required for targeting of the somatic hypermutation process in mice, we examined whether a T cell receptor (TCR) transgene under the control of the immunoglobulin (Ig) heavy (H) chain intron enhancer would be mutated in antigen-stimulated B cells. Hybridomas were established from splenic B cells of mice carrying two copies of the TCR transgene after hyperimmunization with phosphorylcholine keyhole limpet hemocyanin. Northern analysis revealed that all of the transgene-containing hybridomas expressed the TCR mRNA. Multiple somatic point mutations were found in seven of eight endogenous Ig V_H genes examined. In contrast, 29 of 32 TCR genes examined contained no mutations. One potential mutation was seen in each of the three other TCR genes. Our data indicate that although the TCR transgene is expressed in B cells, it is not efficiently targeted by the mutator mechanism. Furthermore, the presence of an Ig H chain enhancer is itself not sufficient for targeting of the somatic hypermutation mechanism.

Somatic hypermutation of Ig genes after antigenic stimulation of B cells is an important mechanism for diversification of the immune repertoire (reviewed in 1, 2). The rate of antigen-induced somatic hypermutation of Ig V region genes has been estimated at up to 10^{-3} /bp/cell generation (3). Although both productively and nonproductively rearranged V genes are targeted, constant regions are spared (4–7). This process appears to be unique to B cells, in that somatic hypermutation does not appear to play a significant role in diversification of TCR genes in T cells.

The molecular mechanism responsible for selectively introducing the characteristic single-base substitutions into Ig V regions and their flanking sequences is unknown. Although gene conversion appears to play a critical role in generation of the immune repertoire in chickens (8, 9) and rabbits (10), a significant role in somatic mutation of Ig genes in mice or humans has not been found (11, 12). The nature and localization of mutations in humans and mice are suggestive of a mechanism involving targeted point mutations (5, 13–17).

We and others have shown that rearranged Ig transgenes can be targeted by the somatic hypermutation mechanism (17–21). The nature and localization of mutations are similar to those seen in endogenous Ig genes (20, 21). The ability of the mutator mechanism to target these Ig transgenes, in-

dependent of their chromosomal location, suggests that they contain any required *cis*-acting elements. In an effort to define potential targeting requirements of the somatic hypermutation machinery, we examined whether a rearranged TCR gene, when expressed in B cells, would be targeted by the mutator mechanism.

Materials and Methods

Mice. Generation of the 2B4 β -L mouse line carrying two copies of the 2B4 β E_H transgene (see Fig. 1) has been described previously (22).

Generation and Screening of Hybridomas. A 2B4 β -L transgenic mouse [(C57BL/6 \times C3H/HeJ)]F₂ backcrossed to C57BL/6) was immunized intraperitoneally with phosphorylcholine (PC)¹-KLH as follows: 0.1 mg in CFA on day -120, 0.1 mg in IFA on day -78, and 0.1 mg in PBS on days -53, -3, and -1, relative to fusion of spleen cells with the myeloma X63-Ag8.653 as described (23). Hybridomas were screened by ELISA for secretion of IgM or IgG, PC-BSA binding (18), and subsequently cloned by limiting dilution. In cases where IgG subclass is indicated, this was

¹ Abbreviations used in this paper: E_H, Ig heavy chain enhancer; L, leader; PC, phosphorylcholine.

determined by DNA sequencing. Determination of whether the hybridomas contained the transgene was carried out using PCR analysis (24). Amplification of 1 μ g of hybridoma DNA was performed with the primers 2B4-1 (5'd[AAACTGCAGGAAGTGTA-ATGAGGAAG]3' at position 28 (25), and 2B4-2 (5'd[GATGGATC-CGCTGAGAACGCGCACGTG]3' at position 604 in 100- μ l reactions containing 50 mM KCl, 10 mM Tris-HCl pH 8.3 (room temperature), 1.5 mM MgCl₂, 0.01% (wt/vol) gelatin, 200 μ M of each dNTP, 50 pmol of each primer, and 4 U *Thermus aquaticus* (Taq) polymerase (Perkin Elmer Cetus Corp., Norwalk, CT). 32 cycles of amplification were performed on a Twin block thermocycler (Ericomp Inc., San Diego, CA). Cycling steps were: denaturation, 1.5 min at 94°C; annealing, 2 min at 55°C; and extension, 3 min at 72°C.

Northern Blot Analysis. Northern blot analysis was performed with 20 μ g of total RNA. To assay 2B4 β E_H transgene expression, blots were probed with a 574-bp fragment encompassing the VDJ region amplified with the primers 2B4-1 and 2B4-2 by PCR (see above), or with a 2.6-kb fragment containing the 2B4 VDJ region isolated from a genomic clone. To monitor expression of the V1 gene of the V_HS107 family, blots were probed with a 189-bp V_H-167-specific fragment (26).

Nucleotide Sequencing. For sequence analysis of endogenous V_H genes, total RNA was prepared by the guanidine isothiocyanate method (27). First-strand DNA synthesis was carried out using the primers X γ 5'd[GGGGCCAGTGGATAGAC]3' and γ 3 5'd[AGGGACCAAGGGATAGAC]3', specific for the CH1 domains of all IgG subclasses or M-CH1 5' d[GCTCTCGCAGGAGAC-GAGGGGGAAG]3', specific for the CH1 domain of IgM. The cDNA was generated in a 10- μ l reaction containing 3–5 μ g RNA, 10 pmol primer (γ 3 + X γ or M-CH1) using avian myeloblastosis virus (AMV) reverse transcriptase (Boehringer Mannheim, Indianapolis, IN) at 42°C for 70 min, then heated at 65°C for 20 min. PCR amplification of the V_H segments was carried out using the degenerate leader region primers MH-SP-ALT.1 5'd[ATG(GA)-A(GC)TT(GC)(TG)GG(TC)T(AC)A(AG)CT(GT)G(GA)TT]3' and MH-SP-ALT.2 5'd[ATG(GA)AATG(GC)A(GC)CTGGGT(CT)-(TA)T(TC)CTCT]3' (28), along with the γ 3 and X γ primers or M-CH1. A mix containing PCR buffer, 50 pmol of each primer, and 4 U Taq polymerase was added to the 10- μ l cDNA reaction to bring the total volume to 100 μ l. A 25-cycle amplification was carried out on a GeneAmp 9600 (Perkin Elmer Cetus Corp.) with denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 60 s. Amplification products were isolated on 1% low-melt agarose (FMC Corp., Rockland, ME) gels, and blunt-end cloned into EcoRV-digested pBluescript KS⁺ (Stratagene Inc., La Jolla, CA). CsCl-banded plasmid DNA or miniprep DNA prepared with Qiagen tip-20 columns (Qiagen, Inc., Chatsworth, CA) was alkali denatured and annealed to KS or SK primers (Stratagene Inc.). Sequencing was performed using the Sequenase kit (US Biochemicals, Cleveland, OH).

To sequence the 2B4 β E_H transgenes, hybridoma DNA (40 μ g) was digested with BamHI, and electrophoresed on 1% low-melt agarose gels to separate the two copies of the transgene L-VDJ region. Agarose blocks corresponding to the 2.8–3.5- and 6.0–16.0-kb regions were excised and melted at 65°C. A 574-bp fragment encompassing the transgene L-VDJ region was amplified by PCR using the primers 2B4-1 and 2B4-2, as previously described (17). 25 cycles of amplification were performed on a GeneAmp 9600 with denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s. The PCR products were cloned and sequenced using the primer 2B4-3 5'd[CACTCAGCAGAGAA-TCG]3' at position 436 of the 2B4 transgene (25), in addition to

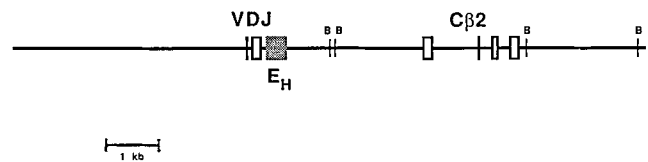


Figure 1. Diagram of the 2B4 β E_H transgene. The rearranged genomic β chain clone contains 4.5 kb 5' sequence and 3.0 kb of 3' flanking sequence. A 687-bp fragment containing the Ig H chain J-C intron enhancer was introduced into a Clal site 3' of the VDJ region (22). B = BamHI.

KS and SK primers as previously outlined. In an effort to distinguish between somatic mutations and Taq polymerase errors, if potential mutations were identified, additional products of the original PCR were examined, or products from a second, independent, PCR reaction were sequenced. Alterations were considered to be actual mutations if they were still present in products of the second PCR reaction.

Results and Discussion

To define potential targeting requirements of the somatic hypermutation mechanism, we utilized the transgenic mouse line 2B4 β -L. The 2B4 β -L line carries a low copy number of a rearranged genomic TCR- β chain construct that lacks the native TCR- β enhancer region (29–31), but contains an Ig H chain enhancer (E_H) element between J and C β 2 (2B4 β E_H, Fig. 1, reference 22). 2B4 β -L mice were hyperimmunized with PC-KLH, and spleen B cells were fused to

Table 1. Properties of the Hybridomas

	PC-BSA	Isotype	Transgene	V1 Expression*
10C8	+	IgM	+	–
10F4	+	IgG	–	ND
1A2	+	IgM	+	–
1A6	+	IgG1	+	+
1E1	+	IgM	+	+
1H2	+	IgG1	+	+
2E2	–	IgG2b	+	–
2F12	+	IgM	+	–
3G5	+	IgG1	+	+
4C2	+	IgG1	+	+
4G6	+	IgG	+	–
4G7	+	IgG	+	ND
5D10	+	IgG1	+	+
8D11	–	IgG	+	–
8E2	+	IgG1	+	+
8G1	+	IgG2a	+	+
3A11	+	IgG	+	–

* Expression of the V1 gene of the S107 family was assessed by probing Northern blots with a V_H-167-specific fragment.

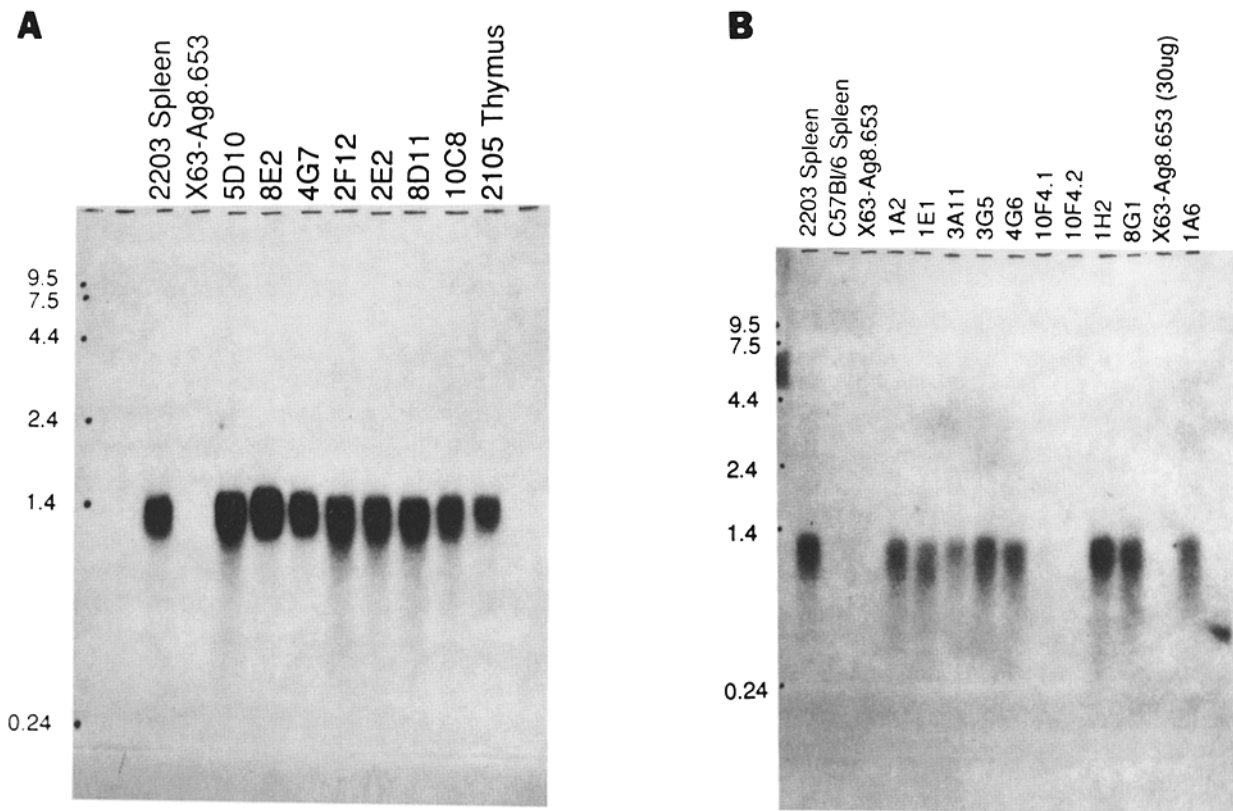


Figure 2. Northern analysis of 2B4 β E_h expression. Blot A was probed with a PCR-generated probe containing the 2B4 VDJ region. Blot B was probed with a genomic clone encompassing the 2B4 VDJ region. RNA from spleen 2203 and thymus 2105 were from 2B4 β -L transgenic mice. Spleen RNA from a C57BL/6 mouse and the myeloma X63-Ag8.653 served as negative controls. Hybridoma clones 10F4.1 and 10F4.2, both derived from the same hybridoma line, do not contain the transgene (see Table I). Based on ethidium bromide staining of the gel before blotting, rRNA quantities were roughly equal in all lanes.

the nonsecreting line X63-Ag8.653 to generate hybridomas. An advantage of this antigen system is that the majority of PC-specific antibodies utilize the V1 gene of the V_HS107 family (32), providing a means of monitoring whether the mutator mechanism has been active in the fused B cells. Furthermore, selection of IgG secreting hybridomas should in-

crease the likelihood of analyzing B cells in which the somatic hypermutation mechanism has been active. Hybridoma clones were screened for IgG and IgM production and binding to PC coupled to BSA. Properties of hybridoma clones selected for study are shown in Table 1. 13 of the 17 hybridomas secreted IgG, and 15 of 17 bound PC-BSA. PCR analysis revealed

	CDR 1						CDR 2																												
	3	4	5	11	12	31	32	35	40	48	50	51	52A	52C	53	54	56	57	58	61	63	70	73	75	76	78	82A	84	87	90	93	94			
V1	AAG	CTG	GTG	TTG	GTA	GAT	TTC	GAG	GCT	ATT	GCA	AGT	AAC	GCT	AAT	GAT	ACA	ACA	GAG	GCA	GTG	TCC	ACT	CAA	AGC	CTC	AAT	GCT	ACT	TAT	GCA	AGA			
1A6	--a	---	---	A	---	A	---	-G	---	---	---	---	---	---	---	---	---	---	-g	---	-a	A	---	---	---	-g	---	---	---	---	---	---	T	---	
1E1	---	t	---	---	---	---	---	-t	---	-c	-T	-c	-t	---	-T	---	---	---	---	---	---	G	---	---	-g	---	---	-G	---	---	-c	---	---		
1H2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	C-g	---	---	---	---	---	C	---	---	---	---	---	---	---	---	
3G5	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	C-g	-G	---	-T	---	-a	A	---	---	-T	---	T	---	T	-Tg	---
4C2	---	---	-a	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	T	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-Cc
5D10	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
8E2	---	---	---	---	---	-T	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
8G1	--a	---	---	---	A	T	---	-T	A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	C	---	---	---	---	---	c-c	

Figure 3. Sequence analysis of endogenous V_H genes. Only hybridomas expressing the V1 gene of the S107 family are shown. Codons of the germ-line C57BL/6 V1 gene (35), in which mutations were observed, are shown. Bases in upper case letters indicate mutations leading to amino acid replacements. Lower case letters represent silent substitutions. (Boxed) CDRs. With the exception of 4C2, all mutations were verified by two independent cDNA and PCR reactions.

that the 10F4 subclones had lost the transgene, and these were eliminated from further analysis. 8 of 16 hybridomas carrying the transgene expressed the V1 gene of V_HS107 based on Northern analysis (data not shown) or RNA sequencing (Fig. 3).

In previous experiments, the addition of an E_H element to a TCR- α chain construct resulted in the expression of this α chain in transfected plasmacytoma (J558L) cells (33). Furthermore, in experiments with transgenic mice carrying this TCR- α chain gene under the control of the E_H, it was expressed in splenic B cells (33). Consistent with these findings, Northern analysis revealed that the 1.3-kb β chain transcript is present in all B cell hybridomas that retained the 2B4 β E_H transgene, as well as in spleen and thymocyte RNAs of 2B4 β -L mice (Fig. 2). Thus, the E_H control element provides for expression of the TCR- β chain construct in B cells, as well as in T cells.

To establish whether the mutator mechanism was active in the B cells before fusion, we examined endogenous V_H

genes expressed by the hybridomas. Primers specific for the CH1 domain of IgM or IgG were used to specifically prime first-strand DNA synthesis. These products were amplified by PCR, cloned, and sequenced (Fig. 3). Only hybridomas using the V1 gene of the V_HS107 family are shown. Since there are only four members in the S107 family (32), mutations of V1 can be scored with greater confidence than V genes in the larger V_H families. In all cases, cloned V1 genes contain the allelic substitutions found in the C57BL/6 germline gene (34, 35). Seven of the eight expressed V1 genes were mutated, with the number of mutations ranging from 3–11. A total of 52 single base substitutions were observed over a total of 2.4 kb analyzed. In a few cases, mutations were shared between V1 genes in different hybridomas. It is conceivable that some (but not all) of these may represent C3H/HeJ allelic differences, as the germline sequence has not been determined. Clearly, the mutator mechanism had been active in the majority of the B cells before fusion.

To determine if the TCR transgene was mutated, a 574-

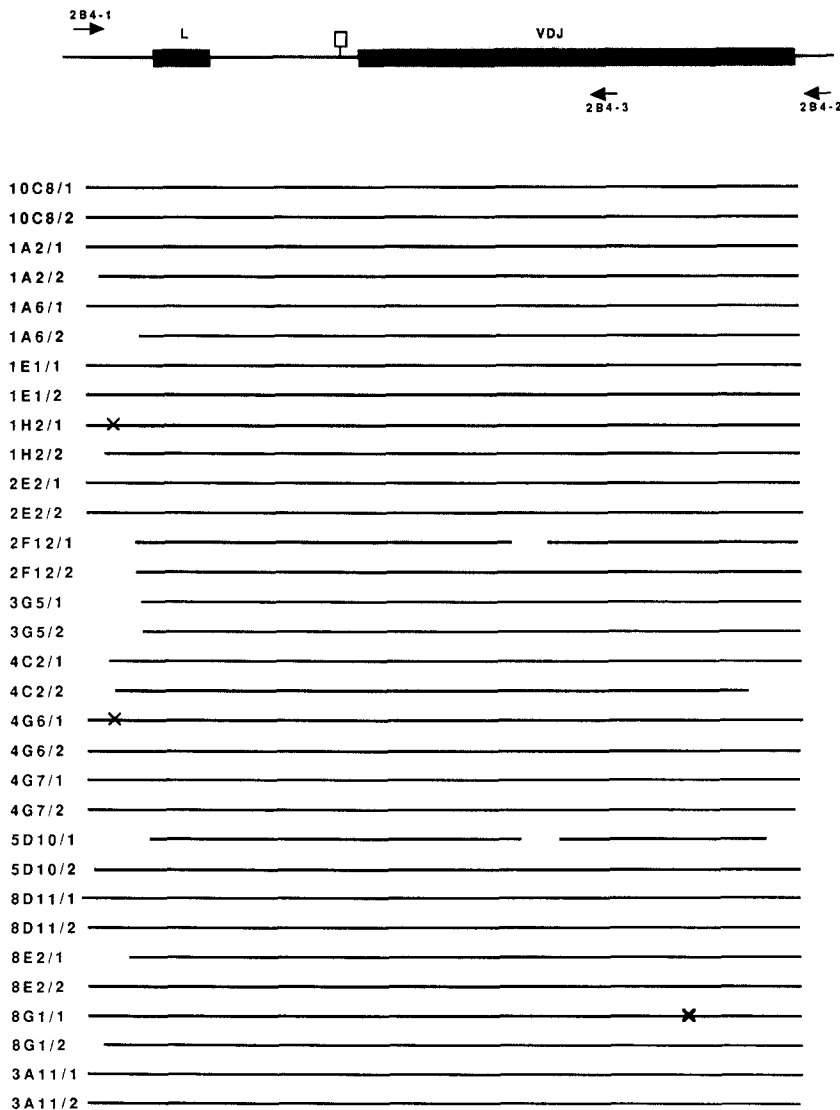


Figure 4. Sequence analysis of 2B4 β E_H transgenes. The amplified region is depicted schematically. (Solid line) The region sequenced. Clones of copy 1 from the hybridomas 2F12 and 5D10 have small sequencing gaps extending from 364–383 and 373–403, respectively. All TCR clones (including those amplified from kidney DNA) contained the following alterations from the published sequence (25): T at 88, A at 116, A at 120, C at 150, as previously noted in a V β 3 clone from C57BL/6 (42). The amplified LVDJ region corresponding to the 7.5-kb BamHI fragment (designated copy 1) in all hybridomas (and transgenic kidney) has a C→T substitution at position 227 ([]) within the L-V intron. (X or X) Alterations from the germline sequence which were confirmed by sequencing products of a second PCR (see text). Clones of copy 1 in hybridomas 1H2 and 4G6 contain an A→G substitution at position 43. Copy 1 in 8G1 has a G→A substitution at position 508.

bp fragment encompassing the 2B4 β E_H LVDJ region (Fig. 4) was amplified from hybridoma DNA by PCR, cloned, and sequenced. This region was chosen because in κ transgenes the analogous region was heavily targeted by the somatic hypermutation process (17, 20, 21). Preliminary analysis of 2B4 β -L transgenic DNA revealed that there are two copies of this target region. In all cases, including transgenic kidney DNA, clones were generated that contained a T at position 227 within the L-V intron, in addition to clones with a C (published sequence, 25) at this position. BamHI digestion and Southern analysis revealed two single-copy bands hybridizing to the 2B4 β VDJ and E_H probes, one at the expected 7.5-kb range and one at 3 kb (data not shown). Whether the smaller fragment has arisen because of a mutation leading to introduction of a new BamHI site (which might have occurred during transgene integration), or whether it represents a partial copy, has not been determined. The orientation of the two copies relative to each other is unknown. Amplification of the separated fragments demonstrated that the larger fragment (copy 1), contains a T at 227, while the smaller fragment (copy 2) has a C at this position. This substitution proved useful in distinguishing the cloned transgene products.

29 of 32 total TCR LVDJ regions analyzed contained no mutations (Fig. 4). Copy 1 cloned from hybridomas 1H2 and 4G6 contained a single mutation (A→G) at position 43, 5' of the L sequence. This identical substitution was seen in products of four other hybridomas, but was determined to be a PCR or cloning artifact, as it was not present in products of another PCR from these four hybridomas. Thus, the significance of this substitution in copy 1 from these two hybridomas is unknown, but we consider it likely that this position may represent a hot spot for PCR errors. Copy 1 cloned from 8G1 contained a single mutation (G→A) at position 508. This substitution was seen in products of three independent PCR amplifications of 8G1 DNA, and thus appears to be a somatic mutation, as it was not present in kidney DNA or the other hybridomas.

Thus, in 17.5 kb of TCR sequence examined (two copies from 16 different hybridomas), only three potential somatic mutations have been identified. Of these, two are an identical substitution found at the same position. If this alteration is somatic in nature, it is conceivable that it occurred early in B cell development, before the onset of somatic hypermutation, and is shared by progeny of this clone.

In any case, the frequency of mutations in the TCR genes

(0.017% if all three substitutions are included) is markedly reduced as compared with that seen in the expressed V_H genes (2.1%). These data indicate that although the TCR β chain gene is expressed in B cells, it is not efficiently targeted by the somatic hypermutation mechanism.

In contrast to B cells, evidence of somatic hypermutation has not been documented in T cells. These data suggest that TCR genes do not contain *cis*-acting elements required for recognition by the mutator mechanism. However, it is not known whether the mutator mechanism is ever activated in T cells. Most likely it is not, and the lack of observed somatic mutations in TCR V genes could be due to the combined absence of *cis*-acting elements and the mutator.

One trivial explanation for the observed lack of targeting of this TCR gene by the mutator mechanism could be an inhibitory effect of the transgene insertion site. However, the ability to demonstrate expression of the transgene suggests that it should be accessible to the mutation process. In multiple cases where κ or H transgenes have been examined, they have functioned as substrates for the mutation process (17–21). These data are consistent with the notion that targeting of Ig transgenes can occur independent of chromosomal location, as long as they are accessible to the mutator mechanism.

An Ig H chain transgene containing only the intron E_H appears to function as a substrate for the somatic hypermutation mechanism (19). However, based on our data, although the Ig E_H element may be required for somatic mutation, it is not sufficient to provide efficient targeting of the mutator mechanism. This is interesting, as truncated κ transgene constructs lacking the 3' κ enhancer (36) do not appear to be targeted for somatic hypermutation (37, 38), even if expressed at high levels (38). This suggested a *cis*-acting role for the 3' enhancer element in the process. Whether it is required for actual recognition of the Ig substrate by the mutator mechanism, or is important for maintaining transcription of the gene while the mutator mechanism is active, is unknown.

Recent analysis of the localization pattern of mutations in endogenous κ and H genes and in multi-copy κ transgenes (17, 20, 39–41) have led to the suggestion that critical *cis*-acting elements may be located 5' to the V genes. Conceivably, the TCR gene differs in this upstream region in a sequence which is essential for targeting the mutation process. The TCR construct used in this study included 4.5 kb of genomic sequence upstream of the leader. It would be interesting to see if the introduction of 5' κ sequences would make this TCR gene a substrate for somatic hypermutation.

We would like to thank Grazyna Bozek for technical help. M. Glymour for her assistance in preparation, and Peter Engler for critical reading of this manuscript.

This work was supported by National Institutes of Health grants GM-38649 and HD-23089, and the Achelis Foundation. J. Hackett, Jr. is a Leukemia Society of America Special Fellow. C. Stebbins is supported by NIH predoctoral training grant GM-07183.

Received for publication 11 February 1992.

References

1. Steele, E.J. 1991. Somatic hypermutation in V-regions. CRC Press Inc., Boca Raton. 185 pp.
2. French, D.L., R. Laskov, and M.D. Scharff. 1989. The role of somatic hypermutation in the generation of antibody diversity. *Science (Wash. DC)*. 244:1152.
3. McKean, D., K. Huppi, M. Bell, L. Staudt, W. Gerhard, and M. Weigert. 1984. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. *Proc. Natl. Acad. Sci. USA*. 81:3180.
4. Kim, S., M. Davis, E. Sinn, P. Patten, and L. Hood. 1981. Antibody diversity: somatic hypermutation of rearranged V_H genes. *Cell*. 27:573.
5. Gearhart, P.J., and D.F. Bogenhagen. 1983. Clusters of point mutations are found exclusively around rearranged antibody variable genes. *Proc. Natl. Acad. Sci. USA*. 80:3439.
6. Pech, M., J. Hochtl, H. Schnell, and H.G. Zachau. 1981. Differences between germ-line and rearranged immunoglobulin V_κ coding sequences suggest a localized mutation mechanism. *Nature (Lond.)*. 291:668.
7. Roes, J., K. Huppi, K. Rajewsky, and F. Sablitzky. 1989. V gene rearrangement is required to fully activate the hypermutation mechanism in B cells. *J. Immunol.* 142:1022.
8. Reynaud, C.-A., V. Anquez, H. Grimal, and J.-C. Weill. 1987. A hyperconversion mechanism generates the chicken light chain preimmune repertoire. *Cell*. 48:379.
9. Thompson, C.B., and P.E. Neiman. 1987. Somatic diversification of the chicken immunoglobulin light chain gene is limited to the rearranged variable gene segment. *Cell*. 48:369.
10. Becker, R.S., and K.L. Knight. 1990. Somatic diversification of immunoglobulin heavy chain VDJ genes: evidence for somatic gene conversion in rabbits. *Cell*. 63:987.
11. Chien, N.C., R.R. Pollock, C. Desaymard, and M.D. Scharff. 1988. Point mutations cause the somatic diversification of IgM and IgG2a antiphosphorylcholine antibodies. *J. Exp. Med.* 167:954.
12. Wysocki, L.J., and M.L. Gefter. 1989. Gene conversion and the generation of antibody diversity. *Annu. Rev. Biochem.* 58:509.
13. Brenner, S., and C. Milstein. 1966. Origin of antibody variation. *Nature (Lond.)*. 211:242.
14. Selsing, E., and U. Storb. 1981. Somatic mutation of immunoglobulin light-chain variable-region genes. *Cell*. 25:47.
15. Manser, T. 1990. The efficiency of antibody affinity maturation: can the rate of B-cell division be limiting? *Immunol. Today*. 11:305.
16. Golding, G.B., P.J. Gearhart, and B.W. Glickman. 1987. Patterns of somatic mutations in immunoglobulin variable genes. *Genetics*. 115:169.
17. Rogerson, B., J. Hackett Jr., A. Peters, D. Haasch, and U. Storb. 1991. Mutation pattern of immunoglobulin transgenes is compatible with a model of somatic hypermutation in which targeting of the mutator is linked to the direction of DNA replication. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:4331.
18. O'Brien, R.L., R.L. Brinster, and U. Storb. 1987. Somatic hypermutation of an immunoglobulin transgene in κ transgenic mice. *Nature (Lond.)*. 326:405.
19. Durdik, J., R.M. Gerstein, S. Rath, P.F. Robbins, A. Nisonoff, and E. Selsing. 1989. Isotype switching by a microinjected μ immunoglobulin heavy chain gene in transgenic mice. *Proc. Natl. Acad. Sci. USA*. 86:2346.
20. Hackett, J. Jr., B.J. Rogerson, R.L. O'Brien, and U. Storb. 1990. Analysis of somatic mutations in κ transgenes. *J. Exp. Med.* 172:131.
21. Sharpe, M.J., C. Milstein, J.M. Jarvis, and M.S. Neuberger. 1991. Somatic hypermutation of immunoglobulin κ may depend on sequences 3' of C_κ and occurs on passenger transgenes. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:2139.
22. Berg, L.J., A.M. Pullen, B. Fazekas de St. Groth, D. Mathis, C. Benoist, and M.M. Davis. 1989. Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. *Cell*. 58:1035.
23. Manz, J., K. Denis, O. Witte, R. Brinster, and U. Storb. 1988. Feedback inhibition of immunoglobulin gene rearrangement by membrane μ , but not secreted μ heavy chains. *J. Exp. Med.* 168:1363.
24. Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, and H.A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science (Wash. DC)*. 239:487.
25. Chien, Y.-h., N.J. Gascoigne, J. Kavalat, N.E. Lee, and M.M. Davis. 1984. Somatic recombination in a murine T-cell receptor gene. *Nature (Lond.)*. 309:322.
26. Storb, U., C. Pinkert, B. Arp, P. Engler, K.A. Gollahon, J. Manz, W. Brady, and R.L. Brinster. 1986. Transgenic mice with μ and κ genes encoding antiphosphorylcholine antibodies. *J. Exp. Med.* 164:627.
27. Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*. 18:5294.
28. Coloma, M.J., J.W. Larrick, M. Ayala, and J.V. Gavilondo-Cowley. 1991. Primer design for the cloning of immunoglobulin heavy-chain leader variable regions from mouse hybridoma cells using the PCR. *Biotechniques*. 11:152.
29. Krimpenfort, P., R. de Jong, Y. Uematsu, Z. Dembic, S. Ryser, H. von Boehmer, M. Steinmetz, and A. Berns. 1988. Transcription of T cell receptor beta-chain genes is controlled by a downstream regulatory element. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:745.
30. McDougall, S., C.L. Peterson, and K. Calame. 1988. A transcriptional enhancer 3' of C β 2 in the T cell receptor β locus. *Science (Wash. DC)*. 241:205.
31. Takeda, J., A. Cheng, F. Mauxion, C.A. Nelson, R.D. Newberry, W.C. Sha, R. Sen, and D.Y. Loh. 1990. Functional analysis of the murine T-cell receptor β enhancer and characteristics of its DNA-binding proteins. *Mol. Cell. Biol.* 10:5027.
32. Crews, S., J. Griffin, H. Huang, K. Calame, and L. Hood. 1981. A single V_H gene segment encodes the immune response to phosphorylcholine: somatic mutation is correlated with the

- class of the antibody. *Cell*. 25:59.
33. Berg, L.J., B. Fazekas de St. Groth, F. Ivars, C.C. Goodnow, S. Gilfillan, H.-J. Garchon, and M.M. Davis. 1988. Expression of T-cell receptor alpha-chain genes in transgenic mice. *Mol. Cell. Biol.* 8:5459.
 34. Clarke, S.H., J.L. Claffin, M. Potter, and S. Rudikoff. 1983. Polymorphisms in anti-phosphocholine antibodies reflecting evolution of immunoglobulin families. *J. Exp. Med.* 157:98.
 35. Kabat, E., T.T. Wu, H.M. Perry, K.S. Gottesman, and C. Foeller. 1991. Sequences of Protein of Immunological Interest. National Institutes of Health, Bethesda. pg. 1416.
 36. Meyer, K.B., and M.S. Neuberger. 1989. The immunoglobulin kappa locus contains a second, stronger B-cell-specific enhancer which is located downstream of the constant region. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1959.
 37. Sharpe, M.J., M. Neuberger, R. Pannell, M.A. Surani, and C. Milstein. 1990. Lack of somatic mutation in a κ light chain transgene. *Eur. J. Immunol.* 20:1379.
 38. Carmack, C.E., S.A. Camper, J.J. Mackle, W.U. Gerhard, and M.G. Weigert. 1991. Influence of a $V_{\kappa}8$ L chain transgene on endogenous rearrangements and the immune response to the HA(SB) determinant on influenza virus. *J. Immunol.* 147:2024.
 39. Weber, J.S., J. Berry, T. Manser, and J.L. Claffin. 1991. Position of the rearranged V_{κ} and its 5' flanking sequences determines the location of somatic mutations in the J_{κ} locus. *J. Immunol.* 146:3652.
 40. Huppi, K. 1984. Germline and somatic diversity of mouse immunoglobulin kappa genes. Ph.D thesis. University of Pennsylvania, Philadelphia. 151 pp.
 41. Lebecque, S.G., and P.J. Gearhart. 1990. Boundaries of somatic mutation in rearranged immunoglobulin genes: 5' boundary is near the promoter, and 3' boundary is ~ 1 kb from V(D)J gene. *J. Exp. Med.* 172:1717.
 42. Gorman, J., K. Minard, N. Shastri, T. Hunkapiller, D. Hansburg, E. Sercarz, and L. Hood. 1985. Rearranged β T cell receptor genes in a helper T cell clone specific for lysozyme: no correlation between $V\beta$ and MHC restriction. *Cell*. 40:859.