EXPRESSION OF T CELL RECEPTOR GENES IN AN ANTIGEN-SPECIFIC HYBRIDOMA AND RADIATION-INDUCED VARIANTS

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The T cell receptor for antigen is similar to that of B cells (Ig) in that there are a very large number of specificities and this repertoire of specificities is clonally distributed. What appears unique about T cell recognition is the ability of T cells to recognize foreign antigen only in association with a product of the MHC (1, 2), whereas antibodies, in most cases, require only antigen. Recent serological and biochemical analyses have shown that the T cell receptor exists on the cell surface as a disulfide-linked multimer of two subunits, α and β (3–7). These peptide chains are generated in a manner similar to Ig in that they are encoded by several sets of gene segments: variable (V); diversity (D), at least in the case of beta chains; and joining (J) regions. These segments rearrange during the maturation of an individual cell to produce a receptor combination unique to that cell lineage (8–13). The rest of the T cell receptor protein consists of a constant (C) region that is itself encoded by three or four separate exons (14–16).

The V regions of the T cell receptor conserve certain residues that are present in both V_H and V_L (17–19). This suggests that their overall structure is similar to that of Ig. Some differences have also been noted (17, 20), whose exact significance remains controversial.

The T cell receptor, like Ig, expresses antigenic determinants of V and C regions. The antigenic determinants of the T cell receptor V regions can be considered as equivalent to the idiotypes of Ig molecules, phenotypic markers of V region genes. T cell idiotypes were identified with antisera or mAbs from syngeneic, allogeneic, or xenogeneic animals immunized with T cell clones (3–7). One such antiidiotypic antibody has been used to select other T cells, which

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were found to have the same H-2 restriction and antigenic specificity and to use the same α and β chains as the original cell to which the antibody had been made (21, 22). This finding indicates that an idiotype can represent a combinatorial determinant formed by the interaction of α and β chains and that the same idiotype can be shared by T cell clones. Also this idiotype can correlate to a particular MHC and antigen specificity of T cell clones.

In this study we investigate the relationship between the idiotype of a KLH-specific, I-E-restricted hybridoma (FN1-18) (23) and the expression of particular V_α and V_β genes. In addition, we have generated a series of x-ray-induced variants of this cell line that no longer respond to antigenic stimulus. We show that in each case this is due to failure to produce a stable transcript of the specific V_β gene, since each of seven independently produced variants have retained their functional T cell receptor genes, yet we could not detect mRNA encoding the FN1-18 β chain V region. This contrasts with the other reports of T cell variants and may indicate a peculiar sensitivity of the β chain locus to x-irradiation.

Materials and Methods

Mice. C57BL/6, BALB/c, (BALB/c × C57BL/6)F_1, AKR/J, P/J, Sm/J, and DBA/1 2–3-mo-old-mice were purchased from The Jackson Laboratory, Bar Harbor, ME. D2.GD, B.10A(5R), B10RSB4, B10RFB2, B10TBR3, and B10RSB2 I-E recombinant inbred strains were kindly donated by Dr. Chella David (Mayo Clinic, MN).

T Cell Hybridomas. These have been described in detail previously (23, 24). In summary, lymph node T cells from KLH-primed $CB6F_1$ mice were fused with the thymoma BW5147, selected in HAT medium and cloned by limiting dilution. They were tested for antigen specificity and genetic restriction elements by their production of IL-2 which was assayed using the HT-2 cell line according to a previously described tchnique (23). The specificities of the hybridomas are summarized in Table I.

Antiidiotype Antibodies. Syngeneic antiidiotype mAbs were produced by immunization of CB6F1 mice with either a KLH-specific, long-term cell line or the FN1-18 hybridoma. The methods used to prepare and to analyze the specificity of these antibodies have been described elsewhere (25). F23-1 mAb specific for V_β chain determinants expressed on ~25% of T cells was kindly provided by Dr. M. Bevan (Scripps Clinic, La Jolla, CA).

Blot Analysis. Northern and Southern blots were performed by standard techniques (26). Probes were labeled either by nick translation (26) or by hexamer priming (27) and hybridized as described (26).

FN1-18 cDNA Library. This was made as previously described (12) in λ gt10 (28) from mRNA prepared by standard techniques. The library was screened with C_α and C_β probes (detailed below). Selected clones were subcloned into the plasmid vector (pUC9) (29) and the V regions sequenced by the method of Maxam and Gilbert (30).

Probes. Probes used in the Northern and Southern blotting experiments were as follows: (*a*) 86T5 (8), this contains D, $J_\beta 3$ (J_β 1.3) and C_β ($C_\beta 1$) and crosshybridizes with all C_β-containing clones; (*b*) A C_α subclone derived from the TT11 Rsa I–Ava II fragment (10); (*c*) V_β FN1-18, a pUC9 subclone of the Eco RI-Xmn-1 fragment of this V region (see Fig. 2); (*d*) V_β 86T1, derived from a thymocyte cDNA clone (8), this represents the V_β of BW5147 and is an Eco RT-Bam HI fragment in pUC9; (*e*) V_α FN1-18, an Eco RI-Hinf I fragment in pUC9 (see Fig. 2); and (*f*) V_α TT11, which represents Eco RI–Alu I fragment (10) of the V_α expressed by BW5147.

Results

MHC Restriction of Various KLH-specific T Hybridomas. The MHC elements by which a series of KLH-specific T hybridomas are restricted have been reported

TABLE I
Genetic Restriction Elements Used To Recognize the KLH by Various T Cell Clones and Hybridomas

Designation	Genetic restriction used for associative recognition	Other reactivities	References
FN1-18	I-E^k DR4 DR7 MT_3	none	23
FN13-21	I-A^d	I-A^b	24
SW2-3	I-A^b	nil	25

previously (23, 24). The results of these studies are summarized in Table I and show that one clone, FN13-21, has an autoreactivity for I-A^b in addition to its H-2-restricted specificity for KLH plus I-A^d. SW2.3 has only one known specificity, for KLH plus I-A^b (24, 25). FN1-18 has no known alloreactivity and recognizes KLH in association with an I-E complementation product as well as with human DR4, DR7, or MT3 (23).

Antigenic Determinants Expressed by the Receptor of T Hybridomas. Several syngeneic mAbs were produced by the immunization of CB6 F1 mice either with an uncloned KLH-specific T cell line or with the FN1-18 hybridoma. B cell hybridomas producing antibodies specific for T cells were selected by RIA using ^{125}I-rat anti-mouse κ chain antibodies as a second reagent. SP2/0, BW5147, $CB6F_1$ thymocytes and a $CB6F_1$ OVA-specific line were used as specificity controls to rule out the possible binding of mAbs to other T cell antigens such as L3T4, T-200, LAF_1 (2 or 3), or the bp54 protein inherited from the BW5147 line as the fusion partner for preparation of the T hybridomas. The results presented in Table II are in agreement with those previously reported (25) and show that all of the T hybridomas bear Thy-1.2 and Ly-1.2, but lack Ly-2.2 or I-A antigens. The S3a.6-18 mAb recognizes a determinant shared by the FN1-18 hybridoma and by the KLH-specific T cell clone A12-11 (Table II.)

To further substantiate the specificity of S3a.6-18 antibody, we carried out competitive inhibition experiments with a series of mAbs specific for L_3T_4 antigen (GK1.5), Thy-1.2 (3DH.12), and Lyt-1.2 (NEI107). As negative controls, we used 10-2-16 (anti-I-A^k) and F23.1, an antibody specific for a V_β determinant that binds to 25% of T lymphocytes of various strains (7), but that does not bind to FN1-18 clones. The data presented in Table III show that these antibodies did not inhibit the binding of S3a.6-18 to FN1-18, suggesting that S3a.6-18 does not interact with L_3T_4, Thy-1.2, or Ly-1.2 antigens associated with the surface of FN1-18 cells.

However, it should be mentioned that we did not succeed in precipitating the receptor of FN1-18 clone with S3a.6-18 from nonionic detergent-solubilized membrane preparation. This can be related to isotype of S3a (μ isotype) since it is known that IgM antibodies have low precipitating abilities.

Effect of S3a. 6-18 Antibody on KLH Recognition. Anticlonotypic antibodies can alter the function of T cell clones and hybridomas, presumably by modulating the T cell receptor and rendering the clones unresponsive to antigen (4, 5). This is the usual effect when the antibodies are in solution, but by attaching the antibodies to beads the effect can be one of activation (5). Previously, Waters et al. (31) have shown that S3a.6-18, an mAb raised against a KLH-specific T cell

TABLE II

Binding of Syngeneic Anticlonotypic mAbs to Various T Cell Clones or Hybridomas

mAb	Isotype	Specificity	FN1-18	FN13-21	SW2-3	BW5147	A12.11	C1.3	D18	CB6/F1 Thymocytes	SP2/0
none			266 ± 92*	312 ± 42	417 ± 52	387 ± 68	689 ± 168	414 ± 28	588 ± 01	1,215 ± 352	306 ± 18
3DH.12	μ	Thy-1.2	33,954 ± 815	27,087 ± 731	21,822 ± 269	888 ± 186	13,489 ± 957	10,046 ± 218	16,802 ± 133	7,780 ± 64	978 ± 154
NE1.107	γ2b	Lyt-1.2	17,948 ± 43	14,402 ± 453	10,655 ± 235	678 ± 25	21,312 ± 391	17,948 ± 200	19,578 ± 991	3,263 ± 103	780 ± 302
NE1.006	μ	Lyt-2.3	1,293 ± 471	1,539 ± 338	933 ± 32	580 ± 102	874 ± 36	647 ± 38	470 ± 212	3,027 ± 99	568 ± 100
10-2-16	γ2a	I-A^k	353 ± 76	776 ± 194	594 ± 139	639 ± 25	693 ± 191	843 ± 77	827 ± 129	1,278 ± 57	508 ± 82
S3a.6-18	μ	KLH line (I)	10,303 ± 868	1,011 ± 344	947 ± 68	897 ± 111	9,544 ± 241	544 ± 140	831 ± 123	1,482 ± 126	970 ± 96

5×10^4 cells were incubated for 45 min at 4°C in presence of 0.01% azide and 3% BSA with 10 μg antibodies. After three washings, the cells were incubated for 2 h at 4°C with ^{125}I-rat anti–mouse kappa mAb (50,000 cpm). Properties of A12.11, C1.3 and D18 have been described elsewhere (31).

* cpm ± SD of triplicates

TABLE III

Inhibition of Binding of ^{125}I-labeled Anticlonotype Antibodies to FN1-18 Hybridoma by Unlabeled mAbs with Various Specificities for Antigens Associated with T Cell Membrane

Unlabeled antibody	Specificity	^{125}I-S3a.6-18
none	—	31,380 ± 1,704
S3a.6-18	FN1-18 and A12.11 KLH T cell clones	3,888 ± 792
GK1.5	L_3 T_4	34,348 ± 1,293*
3DH.12	Thy-1.2	36,716 ± 1,886
NEI-107	Lyt-1.2	34,296 ± 1,352
10-2-16	I-A^k	30,556 ± 558
F23.1	V_β chain	33,258 ± 1,654

5×10^5 cells were incubated for 45 min. at 4°C in presence of 0.01% azide and 3% BSA with 10 μg cold antibodies. After three washings, the cells were incubated for 2 h at 4°C with S3a.6-18 (10^5 cpm/well). In independent experiments, we found that GK1.5, 3DH.12, and NEI-107 bind to FN1-18 cells whereas F23.1 does not.

* cpm – average of triplicates ± SD.

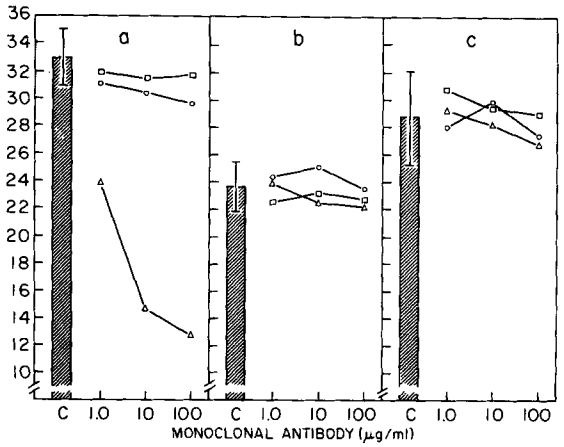


FIGURE 1. Effect of antiidiotype mAbs on IL-2 production by T cell hybridomas. (*a*) FN1-8, (*b*) SW2.3, (*c*) FN13-21. (□) UPC10 (IgG_{2a}), (○) SW2050, (△) S3a.6-18. cpm of 3H thymidine incorporation by 3×10^3 HT-2 cells incubated with 1:4–1:32 dilution of supernatant from cell cultures (3×10^4 T cell hybridomas, 5×10^5 5,000 rad irradiated spleen cells, and 40 μg KLH). Only the results with 1:16 dilution where shown. *C*, control without antibody added.

line, bound to and inhibited both the proliferation and helper function of the KLH-specific clone A12-11 without affecting the functions of two other clones, C1.3 and D18, to which it did not bind. Since we have shown that S3a.6-18 binds to clone FN1-18, we studied its effect on the KLH-specific IL-2 production by FN1-18 and two other clones recognizing KLH with other genetic restriction elements. The data presented in Fig. 1 show that S3a.6-18 inhibits the IL-2 production by FN1-18 but not of the other two KLH specific-clones, SW2.3 and FN13-21.

These results suggest that S3a.6-18 binds to an antigenic determinant of the T cell receptor of FN1-18 involved in the recognition of the KLH-I-E complex.

TABLE IV
Con A–induced IL-2 Production

Clones	^{2}[H]thymidine incorporation	
	none	Con A (5 μg/ml)
FN1-18	605 ± 170*	21,825 ± 688
Variant 11	1,574 ± 258	16,296 ± 148
26	499 ± 53	32,720 ± 892
23	1,186 ± 76	20,564 ± 844
104	1,014 ± 49	12,514 ± 264
106	1,160 ± 192	19,096 ± 528
101	1,122 ± 24	16,416 ± 218
113	1,922 ± 28	18,217 ± 872
116	1,334 ± 154	22,448 ± 404
117	1,198 ± 47	10,595 ± 322

3×10^4 cells were incubated during 48 h in 10% FCS supplemented RPM1 medium and the supernatant (25%) was incubated for 18 h with HT-2 cells. 1 μCi ^{3}[H]thymidine (1 mCi/mmol) was added 12 h before harvesting the cells. Radioactivity was measured in a scintillation counter.

* cpm = mean ± SD of triplicated cultures.

TABLE V
Binding of Anti-Thy-1.2 and Anticlonotype Antibodies to FN1-18 Variants

Clones	FN1-18 variants	
	3DH.12	S3a.6-18
BW5147	886 ± 186	778 ± 224
FN1-18	10,751 ± 1,108	9,302 ± 1,232
Var. 11	11,156 ± 224	5,620 ± 458
26	10,446 ± 354	7,554 ± 294
104	10,442 ± 212	8,882 ± 247
106	10,904 ± 202	11,625 ± 1,197
113	11,982 ± 378	10,763 ± 399
116	10,931 ± 1,694	12,818 ± 1,328
117	10,174 ± 1,879	8,935 ± 1,192

See legend for Table II.

Radiation-induced Mutants of FN1-18. FN1-18 was subjected to 300 rad x-irradiation and surviving cells were cloned by limiting dilution. Clones of cells that grew up were tested for antigen reactivity, and a series of mutants were identified that no longer produced IL-2 in response to antigen but would produce it when treated with Con A (Table IV). Despite this loss of antigen reactivity, studies with the S3a.6-18 showed that in no case was there a significant reduction in binding (Table V). Other workers have found variants of cell lines that have mutations in their T cell receptor genes that are correlated to changes in specificity (32). We found, however, that no IL-2 was produced when FN1-18 variants were incubated with KLH and irradiated spleen cells from various haplotypes of I-E recombinant strains. Thus, the variants did not acquire new alloreactivities or use other I-E complementation gene products as genetic

restriction elements (Table VI). We therefore tried to analyze this nonresponsiveness at the level of the gene.

Characterization of the Active V regions of VN1-18. A cDNA library was made from FN1-18 mRNA, and α and β chain–containing clones were isolated and sequenced. The sequences of both the α and β chain V-(D)-J regions expressed (see below) by FN1-18 are shown in Fig. 2*b*. Both are V regions not previously identified in other T cell clones. We have previously presented the V_α sequence in reference 20; the β chain uses the same J region as 86T1 (8), namely $J_\beta 3$ (J_β 1.3) (14). V_β FN1-18 is very divergent from other V_βs, ranging from 15 to 26% similar at the protein level (data not shown) (20), where mean similarity is 30 ± 11%. The V_α FN1-18 is 24–38% similar (mean, 36 ± 13%) to the other V_α sequences published and uses the same J_α as the T cell clone LB2, which recognizes chicken red blood cells in association with I-A^b (20). Fig. 2*a* also shows the extent of the subcloned probes used in the hybridization studies.

Northern Blot Analysis. Total cytoplasmic RNA from FN1-18, its radiation-induced variants, and FN13-21 were electrophoresed in formaldehyde/agarose gels, blotted onto nitrocellulose, and hybridized to V_α FN1-18 and C_α probes (Fig. 3, *a* and *b*). While all the cell lines express C_α messages (~1.5 kb), only FN1-18 and its variants, but not FN13-21, express V_α FN1-18. In contrast, when blots were hybridized to β chain probes (Fig. 3, *c* and *d*) only FN1-18, but neither the seven variants nor FN13-21, expressed detectable levels of V_β FN1-18 at the mature message size of 1.3 kb. The DJC message (1.0 kb), of unknown function (33, 34), was found in FN1-18 and all its variants except variant 26. It was not present in FN13-21. FN1-18, variants 11 and 26, and FN13-21 were also analyzed for expression of the V_α and V_β BW5147 mRNAs. All were positive, as expected. Fig. 3*f* shows that they hybridized with 86T1 V_β probe, known to detect V_β BW5147 mRNA (18).

Southern Blot Analysis. Genomic DNA from the same cell lines as above and the hybridoma SW2.3 (25) were prepared, as was DNA from the fusion partner BW5147 and liver cells from mouse strains AKR, BALB/c, and C57BL/6. AKR is the control for BW5147, which is an AKR thymoma, and the other strains are controls for the $CB6F_1$ mice, from which the hybridomas were derived. 10 μg of DNA was digested with the restriction enzymes Eco RI (Fig. 4, *a* and *b*) or Pvu II (Fig. 4, *c* and *d*). After electrophoresis and blotting, the filters were hybridized to a variety of probes. Fig. 4*a* shows that V_α FN1-18 exists as a family ($n > 5$) of related sequences in the genome of all three mouse strains, as are the majority of V_α sequences (19, 20).

A 6.6-kb band is present in all the FN1-18 series, but not in the other lanes, and probably represents the functional V_α. Several of the bands present in the C57BL/6 or BALB/c liver cells are deleted in the hybridomas. This is probably due to deletions that occurred during rearrangement of the functional V_α. The pattern of these bands then indicates that the FN1-18 series of variants has not undergone any additional rearrangements and that FN13-21 has undergone a different rearrangement and therefore cannot use the same V_α as FN1-18. SW2.3 has a very similar rearrangement to FN13-21 and may indeed be identical, although this is not certain from this blot. It should be mentioned that SW2.3 was obtained from an independent fusion and uses I-A^b as its restriction element.

TABLE VI

Lack of IL-2 Production by FN1-18 Variants Incubated with KLH and Irradiated Spleen Cells from I-E Recombinant or Mouse Strains with Various Haplotypes

Origin of APC	H-2 locus						Clones								
	K	A_β	A_α	E_β	E_α	D	FN1-18	Var11	Var26	Var104	Var106	Var117	Var101	Var113	Var116
$CB6F_1$	b/d	b/d	b/d	b/d	b/d	b/d	9,778 ± 366*	1,380 ± 82	1,570 ± 56	1,582 ± 72	1,172 ± 361	1,214 ± 219	1,464 ± 92	1,082 ± 252	1,264 ± 380
C57BL/6	b	b	b	b	b	b	1,982 ± 340	1,234 ± 510	1,814 ± 140	1,156 ± 212	1,508 ± 274	1,054 ± 258	1,472 ± 148	1,370 ± 218	1,056 ± 164
BALB/c	d	d	d	d	d	d	1,418 ± 184	1,470 ± 246	1,268 ± 291	1,988 ± 317	1,492 ± 92	1,644 ± 162	1,236 ± 142	1,526 ± 211	1,926 ± 325
AKR	k	k	k	k	k	k	11,942 ± 242	1,478 ± 90	1,822 ± 384	1,830 ± 254	1,332 ± 289	1,774 ± 466	1,802 ± 614	1,202 ± 304	1,632 ± 193
P/J	p	p	p	p	p	p	1,030 ± 376	1,998 ± 284	1,648 ± 122	1,648 ± 212	926 ± 64	1,326 ± 202	1,162 ± 158	1,366 ± 86	1,674 ± 150
Sm/J	v	v	v	v	v	v	976 ± 274	1,360 ± 572	1,666 ± 246	1,814 ± 258	1,634 ± 120	1,684 ± 138	1,034 ± 122	1,746 ± 76	1,922 ± 224
DBA/1	q	q	q	q	q	q	1,724 ± 170	1,946 ± 256	1,300 ± 110	1,755 ± 49	1,156 ± 81	1,542 ± 456	1,254 ± 360	1,754 ± 210	1,080 ± 94
D20GD	d	d	d	d	b	b	1,918 ± 258	1,502 ± 266	1,144 ± 80	1,546 ± 263	852 ± 120	1,460 ± 332	1,282 ± 162	1,542 ± 166	1,360 ± 376
B10.A(5R)	b	b	b	b	k	d	10,510 ± 246	1,542 ± 478	1,072 ± 66	1,868 ± 247	1,366 ± 326	1,292 ± 402	1,142 ± 328	1,066 ± 56	1,436 ± 262
B10.RSB4	s	s	s	s	k	b	1,602 ± 402	1,912 ± 238	1,852 ± 152	1,946 ± 65	1,170 ± 250	1,600 ± 240	1,682 ± 212	1,300 ± 74	1,512 ± 96
$B10.RFB_2$	f	f	f	f	k	b	1,528 ± 96	1,820 ± 338	1,038 ± 62	1,348 ± 207	1,642 ± 504	1,800 ± 154	1,238 ± 494	1,628 ± 40	1,554 ± 52
B10.TBR3	s	k	k	k	b	b	1,184 ± 214	1,858 ± 208	1,120 ± 142	1,698 ± 273	1,276 ± 48	1,334 ± 234	1,112 ± 150	1,322 ± 26	1,098 ± 120
B10.RSB2	s	s	s	s	k	d	834 ± 76	1,630 ± 62	1,426 ± 72	987 ± 90	1,372 ± 102	1,033 ± 291	888 ± 174	1,084 ± 264	1,286 ± 132

The results of ^{3}[H]thymidine incorporation for cultures without KLH are not shown; they were very similar to those of cultures containing KLH. In the use of FN1-18 clone incubated with $CB6F_1$ cells, clone was 1,386 ± 288; with AKR cells 1,418 ± 152; and with B10.A(5R) cells 1,391 ± 258, respectively.

* ^{3}[H]thymidine incorporation by 3×10^5 HT-2 cells incubated with 1:4–1:32 dilutions of supernatant from cell cultures (3×10^4 T cell hybridomas 5×10^5 5,000 rad irradiated spleen cells and 40 μg KLH). Only the results with 1:16 dilution of supernatant are shown. Underlining denotes positive values; all other values are negative.

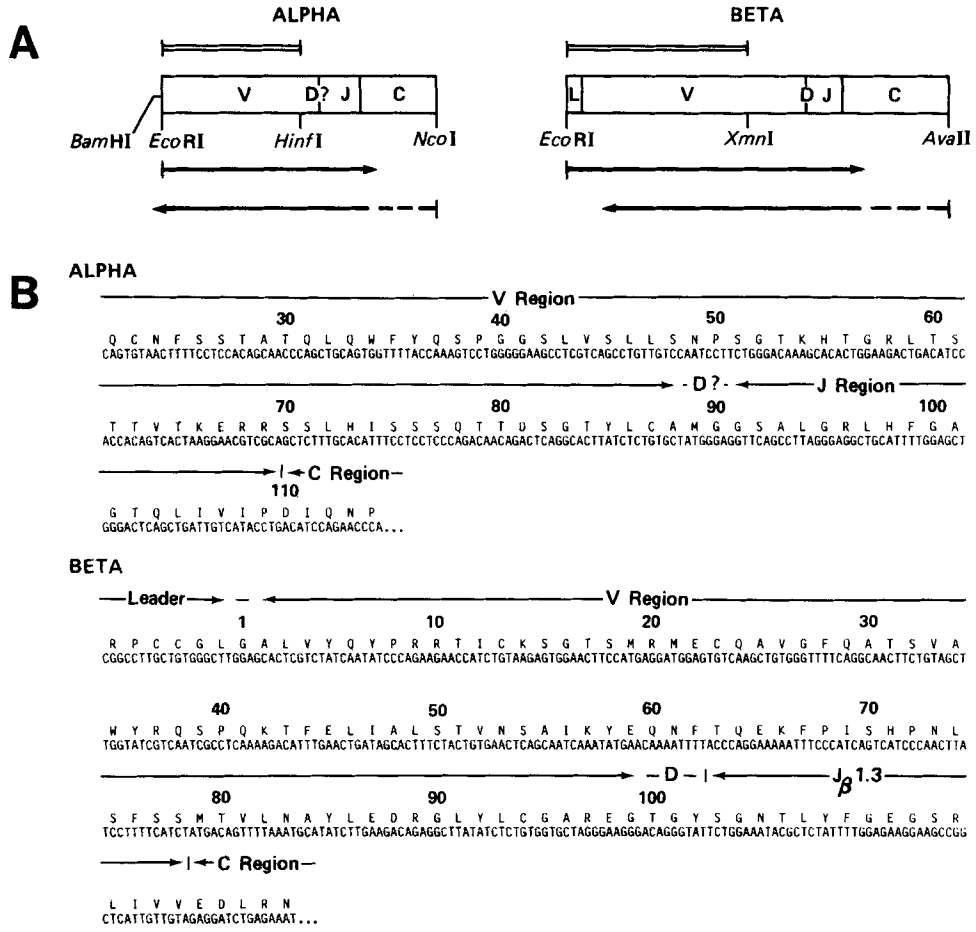


FIGURE 2. (*A*) The variable regions of the α and β chain cDNAs derived from an FN1-18-specific cDNA library were sequenced by the strategy shown in Fig. 2 *a*, using the technique of Maxam and Gilbert (30). DNA fragments were labeled with ^{32}P α-dCTP or dATP using the Klenow (large) fragment of DNA pol I. The Bam HI site shown is from the polylinker of pUC9. The Eco RI sites derive from the oligonucleotide linkers added during cDNA cloning. Also shown is the V region probes (thin open boxes) used in Fig. 3. (*B*) The sequences of V_α FN1-18 and V_β FN1-18 are shown with their predicted protein sequences. These sequences are analyzed in detail in reference 20.

Hybridization with the C_α probe (Fig. 4*b*) shows that all hybridomas contain a hybridizing band at 4.7 kb that is not present in BW5147 or the liver cells, and thus must represent a difference due to rearrangement of one of the α chain chromosomes.

The Pvu II–digested DNAs were hybridized with beta chain probes. Fig. 4*d* shows the hybridization of the 86T1 probe. There are three strong bands in the liver cells at 6.2, 6.0, and 5.8 kb, the usual pattern in unrearranged cells (33, 34), and in BW5147 there are two bands at 6.6 and 5.7 kb. All the hybridomas contain the BW5147 bands. The FN1-18 hybridoma also contains two of the germline bands, which implies that there has been no rearrangement to C_β ($C_\beta 2$) in at least one chromosome. This is confirmed by the fact that a J region from

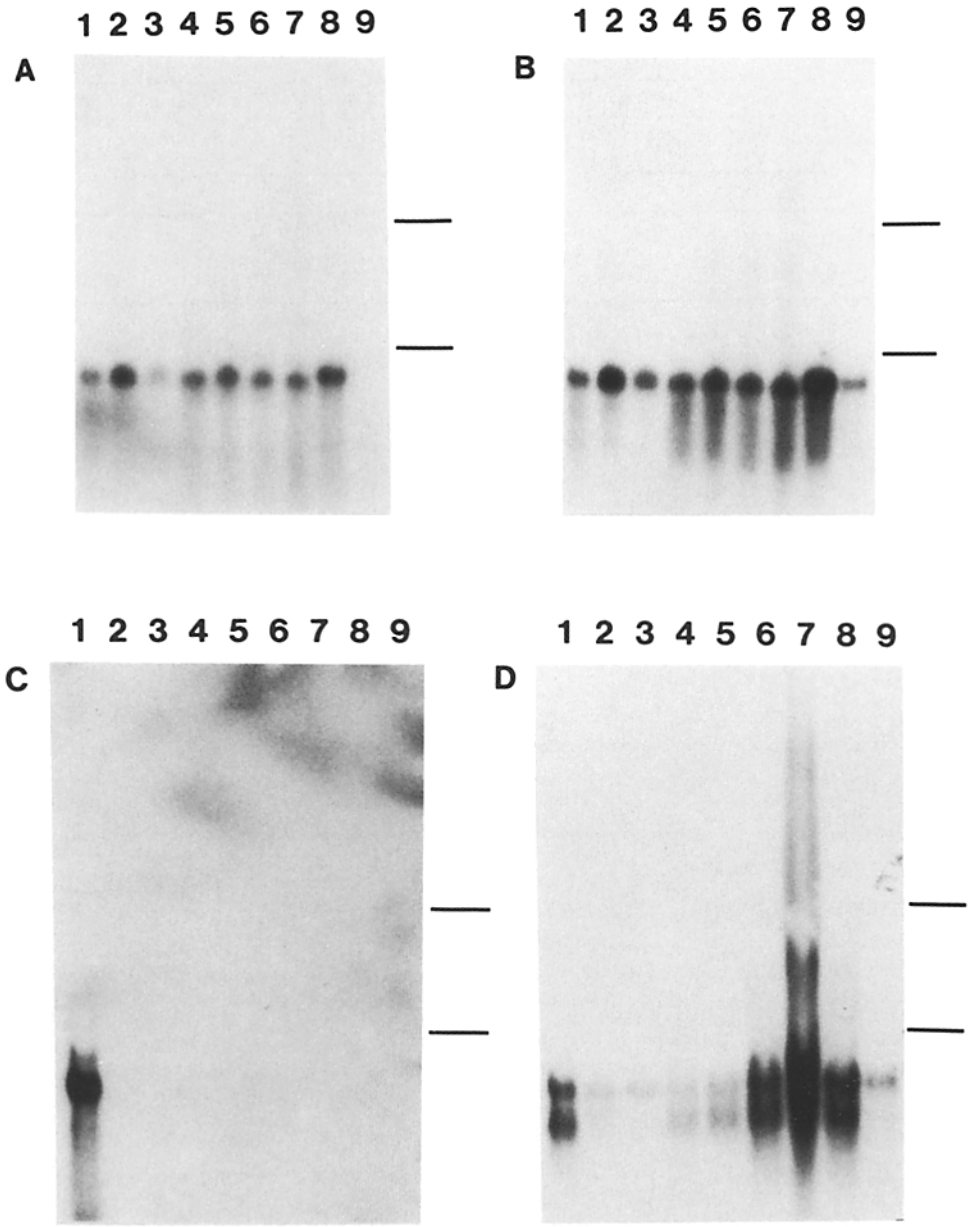


FIGURE 3. Northern blot analysis of mRNA from FN1-18 (lane *1*), variants 11 (lane *2*), 26 (lane *3*), 101 (lane *4*), 106 (lane *5*), 113 (lane *6*), 116 (lane *7*), 117 (lane *8*), and FN13-21 (lane *9*). (*A*) V_α FN1-18 probe, (*B*) C_α probe, (*C*) V_β FN1-18 probe, and (*D*) 86T5 (J_β-C_β) probe. (*E*) shows a longer exposure of lanes *2–5* of *D*. The two arrows show the relative positions of 28 S (~5.0 kb) (*upper*) and 18 S (~1.8 kb) (*lower*) ribosomal RNA. (*F*) V_β 86T1 probe. FN1-18 (lane *1*), variant 11 (lane *2*), 26 (lane *3*), BW5147 (lane *6*).

the 5′ cluster ($J_\beta 3$ ($J_\beta 1.3$)) is used by this cell line (Fig. 3*b*). In addition, FN1-18 has more weakly hybridizing bands at 3.8, 3.2, 2.3, and 2.0 kb, which represent hybridization to the D or J region sequences of 86T5. The two smaller bands

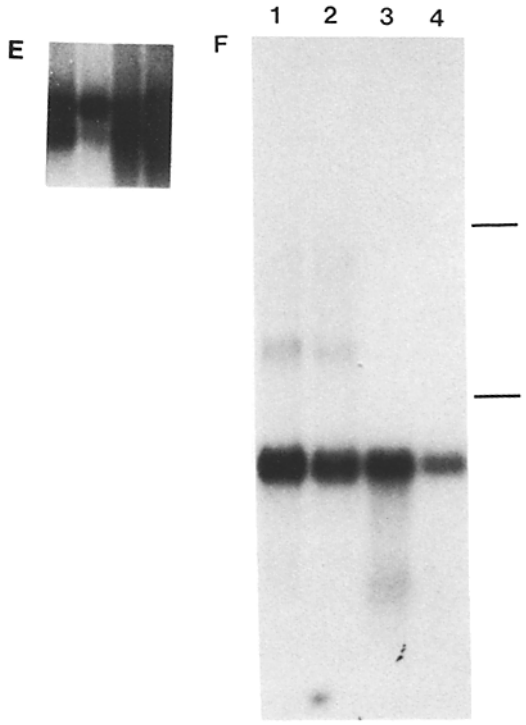


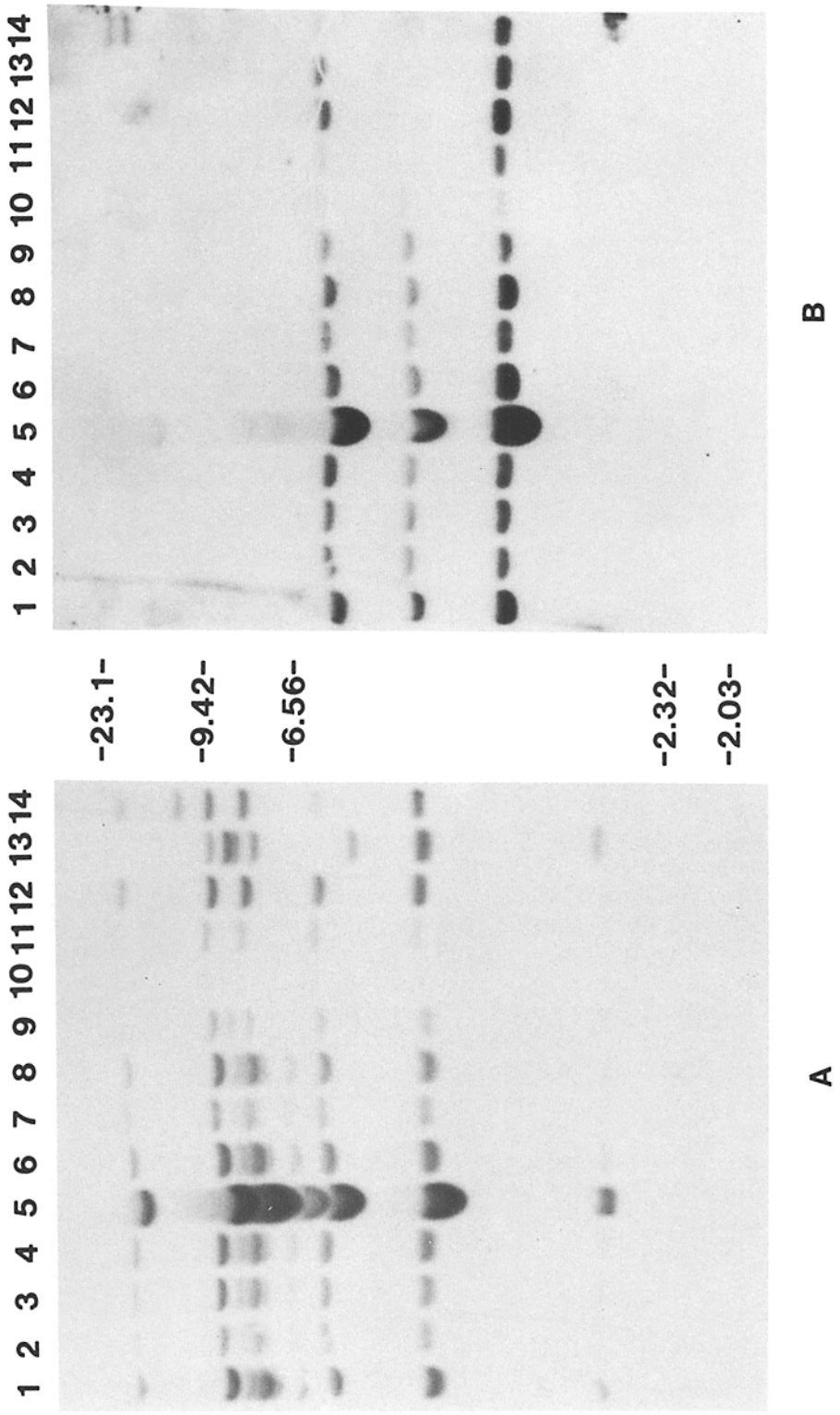
FIGURE 3.

are of germline pattern and thus represent the unrearranged copies of D and J. The 3.8-kb band represents the rearranged VJD band (see below). FN1-18 expresses a DJC 1.0-kb mRNA (Fig. 3*d*). Of all the FN1-18 variants, only variable 26 does not express this mRNA and it is also this cell that has no band at 3.2 kb, the size known to correspond to a rearrangement of D to $J_\beta 3$ ($J_\beta 1.3$) from the restriction map (33). Other than this, the patterns of C_β rearrangement in the variants are identical to FN1-18. The 3.8-kb band corresponds to the copy of $J_\beta 3$ ($J_\beta 1.3$) that is rearranged to V_β FN1-18 (see Fig. 4*c*). FN13.21 and SW2.3 have, as in the α chain, very similar rearrangements, although they are not identical, since SW2.3 has a band at 3.4 kb that is not present in FN13.21.

The V region–specific probe (Fig. 4*c*) indicates that whereas all the liver cells have a single band at 4.3 kb, indicating the unrearranged version of this V_β, only FN1-18 and its variants contain a rearranged version at 3.8 kb. The apparent size difference between FN1-18 and the variants is an artifact of this particular gel. On other blots, the sizes are identical (as in Fig. 4*d*). The lack of a V_β FN1-18 copy in FN13.21 and SW2.3, therefore, indicates that they must use β chain V regions that are downstream of V_β FN1-18 and have lost their copy of it during rearrangement.

Discussion

We have explored the relationships between T cell idiotypes and the expressed T cell receptor V region genes by producing mutated variants of a well-


1 2 3 4 5 6 7 8 9 10 11 12 13 14
B
-23.1-
-9.42-
-6.56-
-2.32-
-2.03-
1 2 3 4 5 6 7 8 9 10 11 12 13 14
A

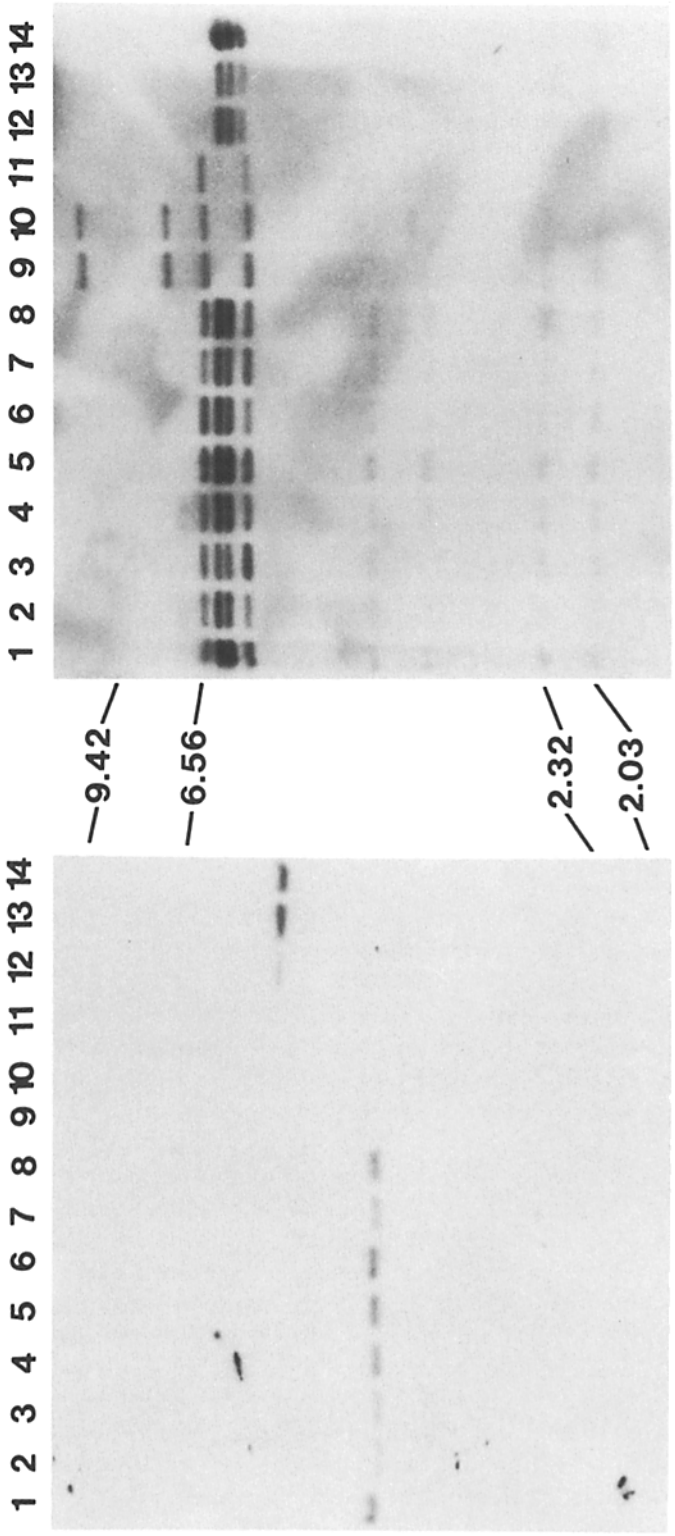


FIGURE 4. Southern blot analysis of DNA from FN1-18 (lane *1*), variants 11 (lane 2), 26 (lane *3*), 101 (lane *4*), 106 (lane *5*), 113 (lane *6*), 116 (lane *7*), 117 (lane *8*), FN13-21 (lane *9*), SW 2.3 (lane *10*), BW5147 (lane *11*), AKR liver (lane *12*), C57BL/6 liver (lane *13*), and BALB/c liver (lane *14*) cut with Eco RI (*A*, *B*) or Pvu II (*C*, *D*). (*A*) V_α FN1-18 probe, (*B*) C_α probe, (*C*) V_β FN1-18 probe, (*D*) 86T5 probe. The position of size markers (in kb) is shown in the margins.

characterized T hybridoma which can no longer respond to antigenic stimuli and have not acquired any new allospecificities, yet can produce IL-2 when activated by Con A. The capacity of variants to produce IL-2 after the binding of lectin to another T cell membrane structure and their ability to express Thy-1.2 and Lyt-1.2 antigens show that the exposure to this dose of x-irradiation did not cause extensive lesions in the genome.

Both the V_α and V_β genes of the parental clone FN1-18 from which the variants had been derived were cloned and sequenced. The β chain V region is, like the majority of V_βs (17), a single copy gene and, like most V_αs (19, 20), V_α FN1-18 exists as a gene family. The FN1-18 variants have lost expression at the mRNA level of their specific V_β gene, which would seem to explain their loss of antigen reactivity. However, they still express antigenic molecule(s) associated with T cell membrane recognized by the S3a.6-18 mAb. This determinant was previously identified on another KLH-specific clone (i.e., A12.11) (31). This antibody certainly recognizes a T cell receptor determinant, because it binds to only FN1-18 and A12.11 among our panel of KLH-specific T cell clones, it inhibits both IL-2 production of FN1-18 cells stimulated with KLH and the helper function of A12.11 clones (31), and its binding to FN1-18 was not inhibited by other mAbs directed against antigenic determinants associated with T cell membrane. The binding of this antibody to variants can be explained by the following.

First, that S3a.6-18 recognizes an α chain determinant and, therefore, a hybrid heterodimer resulting from the assortment of V_α FN1-18 and V_β of BW5147 gene products. This explanation is supported by the presence of a V_β BW5147 message identified with a 86T1 probe in Northern blots in variants.

There is evidence for the occurrence of such a hybrid molecule where a complementation between alpha-BW5147 and another β chain seems to produce a receptor that gives weak anti-IA^b alloreactivity (22). A precedent also exists for a chain-specific T cell receptor determinant. The KJ16 and F23.1 antibodies (35) recognizes a particular V_β found on ~20% of peripheral T cells in most mouse strains (32).

A further possibility is that the mAb may recognize another polymorphic, clonally restricted structure on the cell surface that is important in the T cell recognition, such as a Th equivalent to the γ chain (36). No candidate molecule has been put forward and its existence seems unlikely from other studies (21, 22).

A third possibility is that S3a.6-18 recognizes a clonotypic marker that is not coded for by V_β and V_α genes. Such markers were recently identified with serological reagents by Allison et al. (personal communication).

The findings presented here are important in respect to other workers' studies of T cell receptor loss mutants. Weiss et al. (37) have made variants of the Jurkat T lymphoma by mutation with γ radiation or ethyl methane sulfonate. They were negatively selected for cell surface expression of either T cell receptor or T3, but invariably lost both. These workers have now extended these studies to show that although in all seven cases examined, the T3 δ chain (38) mRNA was present, full-length β chain message was detectable in each case. Interestingly, as we have found, the mutants preferentially lost the β chain rather than the α

chain. Yague et al. (22) describe spontaneous chromosome loss variants where V_α is sometimes lost, but it is likely that this is due to a different mechanism from that occurring in deliberately mutagenized cells.

The mechanism by which x rays produce mutation is still relatively obscure. Malling and de Serres (39), working with *Neurospora*, found that >90% of the 101 ad-3B x-ray mutants that they studied appeared to be single base substitutions, insertions, or deletions. In contrast, a study of x-ray-induced mutants of the *Drosophila* alcohol dehydrogenase gene (40) revealed 20/28 (71%) to be deletions that included neighboring loci. The screen in this case strongly selected for "non-leaky" mutants and thus may have biased the results against point mutations (40).

The FN1-18 variants do not seem to have suffered any gross lesions in the DNA. This seems also to be true for some of the other reported β chain loss variants (41; A. Weiss, personal communication), 4/7 of which were radiation-induced. This implies point mutations or very small deletions rather than large deletions. The mutations would have to be in a region directly or indirectly controlling transcription of V_β FN1-18 mRNA, which must operate in *cis* since the BW5147 beta chain is still transcribed. An alternative explanation is that the x-ray mutations may have occurred upstream of the Pvu II fragment on which the rearranged V_β FN1-18 is found. This would limit the positions of the mutations to >2 kb 5′ to the V region. The mutations induced in the FN1-18 variants must therefore be either small changes in nearby controlling regions of DNA that very strongly decrease the level of transcription or they may be mutations of any size >2 kb upstream of the V_β FN1-18 structural gene. Controlling elements have not been found this far upstream in Ig genes.

Whatever the mechanism, it seems from our studies and others (37, 41) that the β chain is more susceptible to mutation than the α chain. In the *Drosophila* studies mentioned above (40), a clustering of breakpoints of deletions was noted and it may be that the T cell receptor beta chain is particularly susceptible to inactivation by mutagenic agents.

In the β chain loss mutants of Weiss and Stobo (37, 41), although the α chain was not lost, the mRNA was expressed at lower levels than normal. When a β chain loss mutant was transfected with the β chain gene, the level of α chain expression increased back to normal levels and the idiotypic protein and T3 were expressed on the cell surface (41). Thus, there seems to be a regulation of α chain expression by the β chain. Since in development the β chain is expressed before alpha (42–44), it is reasonable to postulate that its presence in the cell signals the α chain to be expressed. In our experiments, we did not observe this lowering of the α message in the FN1-18 beta loss variants since the full-length β chain message from the fusion partner was always expressed.

In conclusion, our results show that the variants obtained from the FN1-18 T cell hybridomas lost their antigenic specificity without acquiring new alloantigen reactivities. The ability to produce IL-2 after lectin stimulation and to bind antiidiotype mAbs specific for the parental clone was preserved. Loss of the ability to recognize the antigen is, in every case, correlated with failure to produce the FN1-18-specific V_β mRNA.

Summary

We have analyzed a series of mutants derived from a KLH-specific, I-E-restricted T hybridoma (FN1-18) which have lost antigen-reactivity while retaining both T cell receptor idiotypic determinants and the ability to respond to Con A. The variants have not gained any detectable alloreactivity, nor is there an obvious lesion in the mutants' beta chain DNA containing the utilized β chain genes. This loss of antigen reactivity is due to a failure of stable production of the specific V_β-containing mRNA. Our results indicate that in FN1-18, the T cell receptor antigenic determinants are most likely carried by the alpha chain alone or by a complementation product of the V_α FN1-18 with the V_β of BW5147. V_β FN1-18 represents a previously undescribed T cell receptor V region.

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