FUNCTIONAL IMMUNOGLOBULIN LIGHT CHAIN GENES ARE REPLACED BY ONGOING REARRANGEMENTS OF GERMLINE V_κ GENES TO DOWNSTREAM J_κ SEGMENTS IN A MURINE B CELL LINE

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Rearrangement of Ig genes proceeds during B cell differentiation in an orderly fashion. First the heavy (H) chain gene is rearranged in pre-B cells. These cells can express cytoplasmic μ chain but no light (L) chain (1). The κ light chain genes rearrange next and if they fail to produce a functional gene on either chromosome the λ light chain genes are rearranged (2). It is generally believed that the expression of an Ig molecule on the cell surface triggers a feed-back mechanism that shuts off the rearrangement process (3, 4). Continuous rearrangement of light chain genes has been reported in some pre-B cell lines transformed with Abelson murine leukemia virus (A-MuLV)[1] (5). Nevertheless, mature B cells that express surface Ig (sIg^+) generally maintain their functionally rearranged Ig genes and remain committed to express antibodies composed of those same H and L chains. Here we show that a sIg^+ B cell can alter its surface receptor by continuing to rearrange its κ light chain locus.

We have previously isolated Ig variants (6) of 38C13, a carcinogen-induced murine B cell lymphoma (7). These variants arose in tumor-bearing animals that were treated with antiidiotypic mAbs. Although a high percentage of the animals were cured, some developed tumors despite the therapy. Analysis of the variant cells revealed that they expressed sIg but failed to react with the antiidiotypic antibody. mAbs were produced that could discriminate between the Ig produced by the parental tumor and by each of the variants. Further analysis showed that the Ig protein expressed by the variants contained identical heavy chains but differed in their light chains. Southern blot analysis showed identical rearrangements at the H chain locus and established that the variants were clonally related. However, the variants differed from each other in their L chain gene rearrangement patterns.

The Ig V region genes expressed by these variants were cloned and sequenced. As expected, the V_H genes were identical in all variants and in the parental 38C13

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[1] *Abbreviations used in this paper:* A-MuLV, Abelson murine leukemia virus; RIV, rate of idiotypic variation; sIg, surface immunoglobulin.

tumor (8). However, the sequences of the V_L genes were all different. Whereas the parental V_L was derived from the $V_\kappa 9$ family and joined to $J_\kappa 2$, the V_L genes of the variants were derived from the V_κ-Ox1 gene family and joined to $J_\kappa 4$. Two of the variants used the identical V_κ gene but differed by the inclusion of variable numbers of additional nucleotides at the V/J joints (8).

These variants were selected, in vivo, from a parental population that contained cells with different rearrangements of the V_κ locus (6). Such variants could have arisen at the pre-B cell stage from a common precursor that had rearranged its heavy chain gene but had not yet chosen a light chain gene. Alternatively the variants could have been generated at the B cell stage by ongoing light chain gene rearrangements.

To distinguish between these possibilities, we have now subcloned the tumor and subjected it to immunoselection in vitro with a toxin-conjugated antiidiotypic antibody. We find that the cloned tumor cell gives rise to variants and these variants can, in turn, give rise to new variants, which all differ in their V_κ genes. The new V region genes are mostly drawn from the V_κ-Ox1 gene family. Interestingly, some members of this family are used repetitively. In this mature B cell tumor, successive V_κ rearrangements occur between members of the V_κ-Ox1 family and progressive downstream J segments. These findings suggest yet another mechanism by which clonal diversity can be generated in Ig-producing cells.

Materials and Methods

Cell Lines. The 38C13 murine B cell tumor and the tumor variants V1-V4 have been described previously (6). The cell lines were maintained in tissue culture as detailed previously (6). New idiotypic variants were derived either by the use of antiidiotype immunotoxin or by subcloning. The immunotoxin was prepared by coupling antiidiotypic mAb S5A8 to the A chain of ricin and was kindly provided by Drs. G. Apell and L. Houston (Cetus Corp., Emeryville, CA). This immunotoxin was used for the selection of variants using methods that were previously detailed (6). Alternatively, cells with spontaneous alteration of idiotype expression, as determined by FACS analysis, were sorted and subcloned. The subcloned cell lines were analyzed again by FACS and new variants were identified by their reactivity with a panel of antiidiotype antibodies (Table I).

Antiidiotypic Antibodies. The generation and characterization of the original syngeneic and rat antiidiotype mAb to the parental 38C13 idiotype protein has been described (9). The isolation of the idiotype proteins produced by the tumor variants V1 and V3 as well as the preparation of syngeneic antiidiotype mAb against these variant idiotypes has also been described previously (6).

Determination of the Rate of Idiotypic Variation. Tumor cells were cloned at limiting dilution in 96-well U-bottomed plates (Costar, Cambridge, MA) in RPMI medium containing 10% FCS. Clones were grown for 5 d then harvested and cytocentrifuged onto microscope slides. Cytospun cells were fixed in cold acetone for 10 min immediately before staining with antiidiotype antibody (S1C5) coupled to horseradish peroxidase (HRP) or with HRP-conjugated polyclonal goat anti-mouse κ (Tago Inc., Burlingame, CA) for 45 min at room temperature. After washing in PBS the slides were developed with AEC (3-amino-9-ethylcarbazole) substrate (Sigma Chemical Co., St. Louis, MO) in *N,N*-dimethyl formamide diluted to 6.7% in 0.1 M acetate buffer, pH 5.2, with 0.03% hydrogen peroxide for 30 min at 37°C. The slides were then washed in PBS and counterstained in 0.1% fast green FCF (Sigma Chemical Co.) for 5 minutes.

Clones stained for idiotype or Ig expression were scored by visual inspection. Clones in which all cells (or the majority of cells) were negative arose from variants present in the original culture and were thus not included in the rate calculations. The rate of idiotypic variation (RIV) was estimated using the P_0 method of Luria and Delbruck (10). The fraction of clones containing no variant cells, P_0, was determined. The mean number of mutational events per

TABLE I

FACS Analysis of 38C13 and Variants

		Target cell lines																		
mAb	Antiidiotype	38C13	V1	V2	V3	V3.1	V4	V4.0	V5	V6	V6.1	V7	V8	V9	V10	V11	V12	V13	V15	V16
S1C5	Anti-38C13	+ + +	−	−	−	+	+ + +	+ + +	−	+ + +	−	−	+ + +	+	−	+ + +	+ +	+ +	+ +	+ +
S5A8	Anti-38C13	+ + +	−	−	+	+	−	−	−	+ + +	−	+ + +	+ + +	+ / −	−	+ + +	−	+ + +	−	−
S3H5	Anti-38C13	+ + +	−	−	−	−	−	−	−	−	−	−	−	+ / −	−	−	−	−	−	−
S4C8	Anti-38C13	+ + +	−	−	−	−	+ / −	+ / −	−	−	−	−	−	−	−	−	−	−	−	−
R7D4	Anti-38C13	+ +	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
R8D5	Anti-38C13	+ +	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
R9A9	Anti-38C13	+ +	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
V1-3E10	Anti-V1	−	+ +	−	−	+ / −	−	−	−	−	+ +	−	−	−	−	−	−	−	−	−
V1-3H12	Anti-V1	−	+ + +	−	−	+	−	−	−	−	+ + +	+	−	−	−	+ +	+ + +	−	+ + +	−
V1-6B12	Anti-V1	−	+ + +	−	−	+	−	−	−	−	+ + +	+	−	−	−	+ +	+ + +	−	+ + +	−
V3-1F1	Anti-V3	−	−	−	+ +	+ +	−	−	−	+ +	−	−	−	−	−	−	−	−	−	−
V3-3A5	Anti-V3	−	−	−	+ +	+ +	+	+	−	+ + +	−	−	−	−	−	−	−	−	−	−
V3-3D6	Anti-V3	−	−	−	+ +	+ +	−	−	−	+ +	−	−	−	−	−	−	−	−	−	−
V3-4D7	Anti-V3	−	−	−	+ +	+ +	+ / −	+ / −	−	+ + +	−	−	−	−	−	−	−	−	−	−
V3-5D3	Anti-V3	−	−	−	+	+	−	−	−	+ / −	−	−	−	−	−	−	−	−	−	−

well, m, was estimated from the Poisson formula $P_0 = e^{-m}$. The variation rate per cell per generation was then calculated using the formula:

$$\text{RIV} = \frac{m(\ln 2)}{N_t - N_0}$$

where N_t was the average number of cells per clone, and N_0 was the number of cells initiating the clone, in this case 1. Even if some clones arose from two or three cells, this would not significantly effect the calculation of RIV, since N_t was three to four orders of magnitude higher. In addition, since only the presence or absence of variants in a clone is scored, it is irrelevant whether they grow faster or slower than the parental cells.

The variance (var) of RIV and the coefficient of variance (CV), a measure of the precision in the estimate of RIV, were calculated as described by Li et al. (11):

$$\text{var(RIV)} = \frac{1 - P_0}{CN_t^2 P_0}$$

where C is the total number of clones screened, and

$$\text{CV} = \frac{\sqrt{\text{var(RIV)}}}{\text{RIV}}$$

Sequencing of V_κ Genes. Total RNA was extracted from the tumor variants using the guanidinium thiocyanate method (12), poly(A)$^+$ mRNA was isolated by oligo(dT) chromatography (13). V region cDNA was synthesized using a C_K primer and reverse transcriptase, made double stranded by the method of Gubler and Hoffman (14) and inserted directly into the Sma I site of m13mp19 as detailed (15). The m13 libraries were screened with a V_κ gene (8). Positive clones were identified and single-stranded templates were sequenced (16).

Results

Primary, Secondary, and Tertiary Idiotypic Variants Can Be Derived from a Cloned sIg$^+$ B Cell Tumor. To test if light chain heterogeneity could be generated as an ongoing process in this fully differentiated B cell, the 38C13 tumor was repetitively cloned and immunoselected in vitro (Fig. 1). These derivative clones were then analyzed with a panel of mAbs that react with different idiotypic determinants (Table I).

At the start of the experiment a subclone of 38C13 cells was expanded and then treated with an immunotoxin made from the antiidiotype antibody S5A8 (6). The cells that eventually grew were mostly sIg$^-$ however, some of the cells expressed surface Ig (sIg$^+$). When analyzed with a second anti-Id antibody, S1C5, these sIg$^+$ cells contained two separate populations, as shown in Fig. 2. Prototypes of each of these two cell populations were then isolated by subcloning, giving rise to tumor variants V4.0 and V6 (Fig. 1). The reactivity pattern of tumor variant V4.0 with a panel of antiidiotypic mAbs (Table I) was identical to that of the previously, and independently, isolated variant V4 (6). Surprisingly, the tumor variant V6 retained reactivity with anti-Id antibody S5A8, despite the fact that it was selected by exposure to the S5A8 immunotoxin. This tumor variant, V6, was then reexposed to a 10-fold higher concentration of the S5A8 immunotoxin and gave rise to the variant V6.1. This second generation variant exhibited a reactivity pattern different from V6 but identical to that of the independently derived tumor variant V1 (Table I), hence the nomenclature. These common reactivity patterns between independently de-

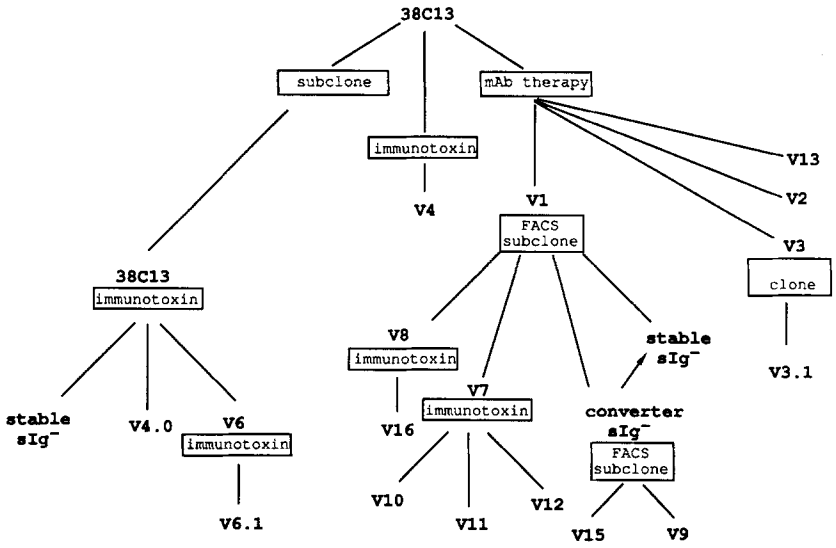

FIGURE 1. Genealogy of 38C13 cell variants. Idiotype variants of the 38C13 tumor were selected as indicated. Antiidiotypic antibody S1C5 was used for mAb therapy in vivo. Antiidiotypic antibody S5A8 was conjugated to ricin A chain and this immunotoxin was used for selection in vitro. In addition, cell lines that demonstrated alterations in their FACS profiles were sorted and subcloned as indicated.

rived variants are explained by the nucleic acid sequence analysis of their V_K genes (see below).

Second and third generation variants were derived from the tumor variant V1, which had been previously isolated by immunoselection in vivo with the antiidiotypic mAb S1C5. To begin this experiment the variant V1 was subcloned and grown in culture for ~3 mo. The resulting cell population was then reanalyzed for idiotype expression and demonstrated to be heterogeneous. Sorting and subcloning of these cells resulted in the isolation of the tumor variants V7 and V8 (Fig. 1 and Table I). Some of the clones demonstrated a unique "converter" phenotype. That

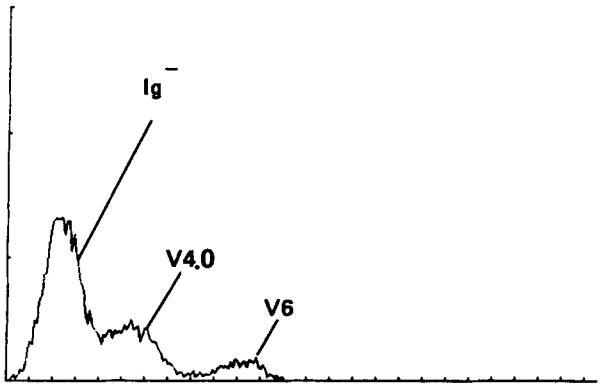

FIGURE 2. FACS analysis of immunotoxin resistant 38C13. Subcloned 38C13 cells were treated with antiidiotype immunotoxin (S5A8-ricin A chain). Resistant cells were stained with a different antiidiotypic antibody S1C5 and analyzed with the FACS. The resistant cells were predominantly sIg^-. The sIg^+ cells showed two different levels of staining intensity. Variants V4.0 and V6 were isolated by subcloning cells from these two cell populations, as indicated.

is, from a population that was >95% sIg^- when stained initially after recloning, the cell population became progressively more sIg^+ with time. This change was detected as early as 10 d in culture. As the subclones converted to become sIg^+, reactivity with some antiidiotype mAbs was detected. This allowed the sorting and cloning of the tumor variant cell lines V9 and V15 (Fig. 1 and Table I). Third generation variants (V10, V11, V12, and V16) were obtained from tumor variants V7 and V8 (Fig. 1) by selection in vitro with the immunotoxin.

Rate of Generation of Idiotypic Variants. The rate at which idiotypic variants were being generated in vitro was determined by fluctuation analysis. Individal subclones of 38C13 were grown for 5 d to an average of 8859 cells. 58 of these colonies were stained for idiotype expression and 53 were found to contain some Id^- cells. Using the P_0 method of Luria and Delbruck (10), the rate of idiotypic variation was estimated to be 1.92×10^{-4} per cell per generation (Table II).

Since cells that do not stain with the antiidiotype antibody could be either Ig^+/Id^- or simply Ig^- variants, we also examined the rate of generation of Ig loss variants in the 38C13 cell line. Of 41 colonies analyzed with an antibody directed against the murine κ light chain, 22 contained some negative cells. As shown in Table II, the resulting rate of κ chain loss was estimated to be 6.01×10^{-5} per cell per generation.

Idiotypic Variants Are Generated by Continuing Rearrangement of κ Light Chain Genes. Messenger RNA was isolated from the tumor variants and cDNAs for their expressed V_κ genes were cloned and sequenced. Fig. 3 contains the sequences of transcripts from the sIg^+ variants V6, V6.1, V8, V7 and from the sIg^- variant V2. The nucleotide sequence of the V2 transcript was out of reading frame beginning at the exon junction within the leader region at codon −4 (not shown in the figure). Thus, it was probably a splicing mutant. TII is a second nonfunctional transcript that was isolated from V6, V6.1, and V7. The TII transcript has an additional base at the V/J joint (the boxed A in Fig. 3). This causes the J and C region to be out of the appropriate reading frame. All these sequences are displayed along with certain previously published members of the V_κ-Ox1 germ line gene family (NQ10 [17], R2, H3 and H6 [18]).

A. Successive Variants Express New V_κ Genes Drawn Predominantly from the V_κ-Ox1 Family.

TABLE II

Variation Rate Measurements in the 38C13 B Lymphoma Cell Line

	Id^-	κ^-
Total clones (C)	58	41
Initial cell no. (N_0)	1	1
Average final cell no. (N_t)	8,859	8,859
Clones containing variants	53	22
Clones containing no variants	5	19
P_0	0.0862	0.4634
m	2.45	0.77
RIV	1.92×10^{-4}	6.01×10^{-5}
var(RIV)	2.33×10^{-9}	3.60×10^{-10}
CV	0.25	0.32

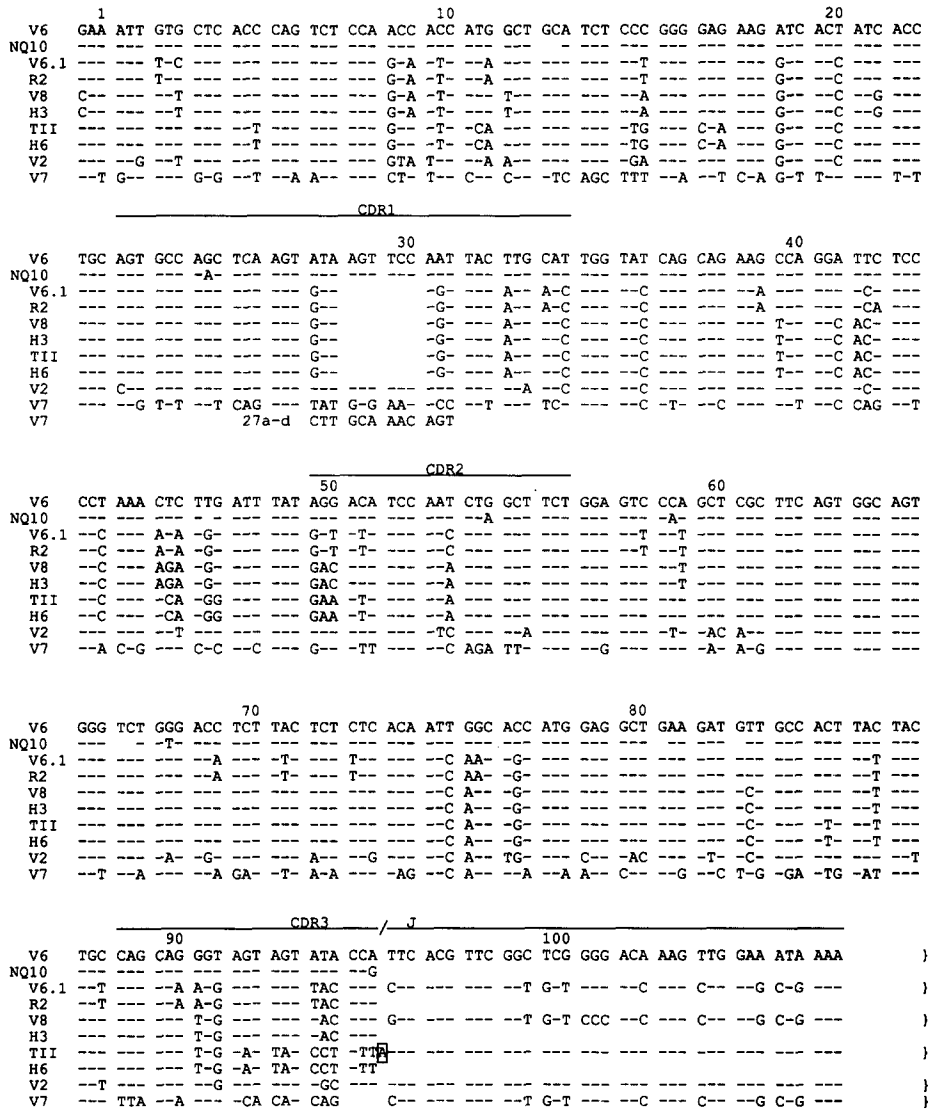

FIGURE 3. Nucleotide sequences of V_K DNA. The nucleotide sequences are numbered according to Kabat et al. (35) and grouped in codons. The complementarity determining regions (CDR 1-3) are indicated, as well as the J regions used. NQ10 (NQ10.4.61) is a previously reported mRNA sequence (17); R2, H3, and H6 are previously reported BALB/c germline sequences (18) of the V_K-Ox1 gene family. The boxed A at the V/J joint in the TII (transcript II) changes the reading frame of the J and constant regions.

Each of the variants expressed V_K genes that were different from that of the 38C13 cell from which they were all derived (Fig. 4). The striking finding was that all but one of these newly expressed V_K genes was a member of the V_K-Ox1 family (Fig. 3). The V6 transcript and that of our previously published V3 and V4 variants (8) used a V_K-Ox1 gene that was almost identical to the one used by hybridoma NQ10.4.61 (17). The V6.1 transcript and that of our previously published V1 variant

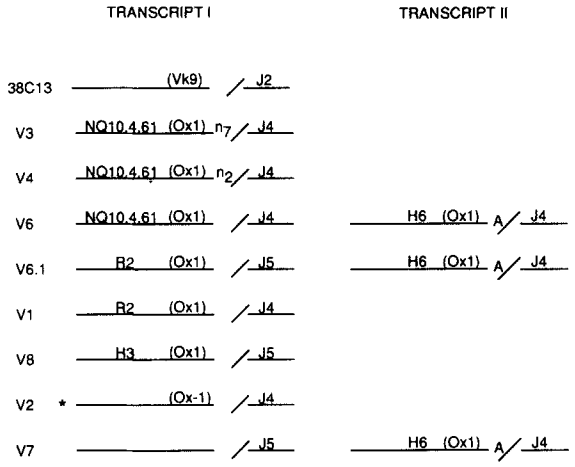

FIGURE 4. Summary of transcripts. The transcripts expressed by 38C13 and all of the tumor variants that have been sequenced to date are given, including data obtained previously for V1, V3, and V4 (8). Repeated usage of the various V_κ-Ox1 gene family members as well as the J region used by each of the transcripts is indicated. The asterisk indicates that the V2 transcript is out of the appropriate reading frame (starting shortly after the leader exon); the A at the V/J joint of transcript II changes the reading frame of this transcript as well. The extra nucleotides (n_n) at the V/J junction are given for the V3 and V4.

(8) used a V_κ gene that was almost identical to the R2 (18) germline gene. The V_κ gene used by the V8 transcript was identical to the H3 gene (18). The V_κ gene of TII, the second nonfunctional transcript isolated from V6, V6.1, and V7, was identical to the H6 gene (18). The V2 transcript, also nonfunctional, used still another member of the V_κ-Ox1 family, but no germline version of this gene has been reported. Only V7 expressed a V_κ gene that was not a member of the V_κ-Ox1 family. It had a long CDR1, with four extra amino acids 27 a-d (35) (Fig. 3). This gene was most homologous to the V_κ gene isolated from the myeloma W3129 (19).

Although the identical V gene was expressed by V3, V4, and V6 and the identical V gene was expressed by V6.1 and V1, these were all independent rearrangement events. This was evident because of the variations in the V/J joints or in the choice of J segments, as indicated in Fig. 4. On the other hand the rearrangement that gave rise to the nonfunctional transcript TII was common to variants V6, V6.1, and V7 and tied these three variants to a common precursor cell. Since V6.1 was derived directly from V6 and since they shared a common nonfunctional transcript TII, the functional V_κ gene NQ 10.4.61 joined to J4 in V6 must have been replaced by a subsequent rearrangement of the R2 gene to the downstream J5 segment to give rise to V6.1.

B. Downstream J Regions Are Used in the Second and Third Generation Variants. The 38C13 V_κ gene was joined to J2 while all of the variants derived from this tumor used either the J4 or the J5 gene segments. Analysis of second generation variants that gave rise, directly, to third generation variants showed that these second generation variants were using the J4 for their active transcript (V1 and V6). By contrast, the third generation variants (V6.1, V7, and V8) all used the J5 for their active transcript (Figs. 3 and 4).

Discussion

The murine 38C13 B cell lymphoma has been used as a model system to study antiidiotype therapy (9). This B cell tumor expresses sIg protein and responds to therapy directed against the idiotypic determinants. In this respect it is similar to human B cell lymphomas. We have previously found that the Ig genes expressed

by certain human B cell tumors undergo somatic point mutation (20). In a search for an in vitro system that exhibits such Ig gene point mutation, we analyzed the tumors that had escaped anti-Id therapy in this mouse model. However, analysis of the 38C13 tumor variants revealed that they ceased to react with the antiidiotypic antibodies not because they had undergone somatic mutation but because they expressed new V_κ genes (8).

The question then arose whether these tumor variants were present in the parental population and were merely selected by the anti-Id antibody therapy. Analysis of the parental population revealed heterogeneity at the V_κ locus and it was possible that the variants arose during some early stage in the evolution of this tumor. We therefore subcloned the tumor and followed the V_κ locus in these clones. Here we demonstrated that subclones of this tumor could give rise to new tumor variants and that these variants could give rise to subsequent variants. For example, the V6 tumor was derived from the subcloned 38C13 and the V6.1 tumor was derived, in turn, from the V6 tumor (Fig. 1).

Continuous rearrangement of V_κ genes has been previously observed in A-MuLV-transformed pre-B cell lines (5). This phenomenon was documented by studying the rearrangements of the V_κ genes by Southern blot analysis. However, the actual V_κ and J_κ genes that were involved in these new rearrangements were not identified. Other groups have described multiple rearrangement events that had occurred during the differentiation of B cells before the formation of an active V_κ allele (21–23). However, in these examples V_κ gene rearrangement ceased once a functional gene was produced.

The continuously rearranging A-MuLV-transformed cell lines are pre-B cells in which the enzymes involved in the V/J recombination are still active (24). A plasmid substrate has recently been used to test cell lines for the presence of an active recombination system (25). As expected, pre-B cells regularly exhibited such activity, but in addition, one mature B cell line, BALB-1427, was found to exhibit an active recombinase.

As we have shown here, the 38C13 B cell tumor and its various variants undergo continuous rearrangement of their Ig V_κ genes. These rearrangement events are restricted almost exclusively to a single V_κ gene family, whose members are used repetitively by independent tumor variants (Fig. 4). Biased usage of V_κ genes of the same family has been previously described (23, 26). It is possible that in the 38C13 tumor an initial functional rearrangement event brought a particular set of upstream V region genes closer to the J region. Such proximity to the J genes could enhance the usage of certain V_κ genes, similar to that described for proximal V_H genes in early B cell development (2). This might explain the repeated usage of the V_κ gene family members in the new rearrangements that occurred in the 38C13 tumor variants. If this explanation for the repetitive use of the V_κ-Ox1 family is valid, then it would imply that the members of this family used here are clustered.

Somatic point mutation does not occur in this B cell tumor. We know this because the genes expressed by C_3H tumor cells were either almost identical or identical to germline genes previously sequenced from BALB/c mice (18). Moreover, repetitive isolates of the expressed genes were identical to each other. Similarly, the V_H gene expressed by the parental tumor and its variants is identical to a germline gene, V_H11 of NZB mice (27).

The use of the same V_κ gene in three independent variants points out still an-

other interesting feature of these light chain gene rearrangements. The V/J joint in variant V4 contains two extra nucleotides and also is missing one nucleotide from the 5′ end of the J segment. Likewise, the light chain gene of the V3 variants has seven extra nucleotides at the V/J junction (nucleotides missing from the 5′ end of the J segment bring the transcript back into the proper reading frame) (8). Long light chain transcripts containing members of the V_κ-Ox1 family have previously been observed and are assumed to be the products of independent genes (28). By analogy it is possible that these three variants express independent genes. But it is more likely that the extra nucleotides are N nucleotides similar to those seen at $V_H/D_H/J_H$ junctions (2). N nucleotides have only rarely been reported in light chain genes both in mouse (29) and in human (30). It has been suggested that N nucleotides are the products of the enzyme terminal deoxynucleotide transferase (TdT) (31). We have previously shown that the 38C13 cells express TdT mRNA (8).

Despite the repeated usage of the same V region gene, the inclusion of extra nucleotides at the V/J junction created Ig proteins that differed from each other by one to three amino acids. Such changes caused marked differences in reactivity of some of the Ig products with a panel of antiidiotypic antibodies (Table I). However, no changes in the reactivity of the V1 and the V6.1 were detected, even though these two variants (which used the identical V_κ gene but different J regions) also differed from each other by three amino acids. Thus, the epitopes recognized by some of the antiidiotypic antibodies were dependent upon the amino acids at the V/J junction.

The parental 38C13 used the J2 gene segment. The next generation variants used J4, the next functional downstream J region in the mouse. As new variants arose they always used J region genes that are still further downstream. For example, the V6.1 tumor used J5 and the tumor from which it was derived, V6, used the J4 gene. Both of these variants were shown to express an identical aberrant transcript (TII in Figs. 3 and 4). Therefore, in this case, the allele that underwent the tertiary rearrangement can be clearly identified.

Some variants of the 38C13 tumor were derived by selection with an antiidiotype immunotoxin. However, others arose spontaneously during passage in vitro. As an example, the V1 variant when left in culture for 3 mo developed new variants. The rate at which variants of 38C13 were being generated in vitro was estimated to be 6.01×10^{-5} per cell per generation for Ig^- variants and 1.92×10^{-4} per cell per generation for Id^- variants (which includes Ig^- as well as Ig^+/Id^- cells). These rates are comparable to those reported by Wabl and co-workers for V region somatic mutation (32), suggesting that continued κ chain rearrangement may represent another source of idiotypic variation that could result in escape from antiidiotype therapy.

The rate of variant generation was measured for the parental 38C13. Some cell lines derived from the V1 variant and initially exhibiting a sIg^- phenotype converted to sIg^+ after as little as 10 d of culture. Other semiquantitative experiments (data not shown) indicate that a surface Ig^- state (converter sIg^- phenotype, Fig. 1) can be an intermediate step in the generation of variants. This observation is concordant with the proposed mechanism of feedback by the Ig protein on new V_κ rearrangements. Indeed it may be that a sIg^- state is an obligatory step in the induction of the new rearrangement process, but this has not been proven.

In human B cell tumors, escape from antiidiotype therapy was found to be explained by a known mechanism for generation of diversity in Ig genes: somatic point

hypermutation. In the mouse tumor described here, escape from antiidiotype therapy was due to a different mechanism: continuing light chain rearrangement. We have recently observed a similar process in the λ locus of a human B cell line, derived from a large cell lymphoma (33). In addition, secondary rearrangements of the TCR V_α genes to downstream J_α segments have recently been shown to replace preexisting and functional V_α/J_α genes in a T cell line (34). It is possible that this is a general mechanism for the generation of Ig and TCR diversity that has not been previously appreciated. This process can be detected only when the progeny of single cells can be recovered and analyzed, such as in cell lines. The study of cells participating in a normal immune reponse does not easily lend itself to such an analysis.

Summary

A murine B cell lymphoma (38C13) was subjected to immunoselection with mAbs directed against the idiotypic determinants of its cell surface Ig. Variants emerged with altered Ig receptors containing identical heavy chains but different light chains. The functional light chain genes in these variants were composed of V_κ segments drawn from the V_κ Ox-1 family, which had replaced the V_κ gene expressed by the parental tumor by rearranging to downstream J_κ segments. Rearrangement at the κ locus continued to occur spontaneously, giving rise to secondary and tertiary variants at a rate of 1.9×10^{-4} per cell per generation. Variants were isolated that had ceased production of surface Ig but went on to rearrange again and to become surface Ig^+. The Ig^- state may be an intermediate step providing a stimulus for continued rearrangement. This process provides an additional mechanism for generating diversity within B cell clones and expands the use of the available repertoire of Ig genes.

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