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Somatic hypermutation of immunoglobulin κ may depend on sequences 3′ of C_κ and occurs on passenger transgenes

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We have compared the pattern of somatic mutation in different immunoglobulin κ transgenes and suggest that an element(s) located between 1 kb and 9 kb 3′ of C_κ is necessary for somatic hypermutation of the antibody V gene. The sequences of transgenic and endogenous Ig V regions were determined in antigen-specific B cell hybridomas specific for 2-phenyloxazolone from independent lines of hyperimmunized transgenic mice. We analysed somatic mutation of the transgene both in hybridomas in which the transgenic κ chain contributes to the antigen combining site as well as in hybridomas in which the transgene is a passenger with the expressed antibody being composed of endogenously-encoded heavy and light chains. In both cases, nucleotide changes in the transgene are correctly targeted to the V region and are absent from the C region. They accumulate at a similar rate to that in the endogenous Ig genes within the same cell and we find that, irrespective of whether or not the transgene κ is directly selected by antigen, somatic mutation occurs at a similar rate and involves only single base substitutions. Furthermore, the pattern of mutations in passenger transgenes gives information about the intrinsic sequence specificites of the somatic hypermutation mechanism.

Key words: antigen selection/B cell/Ig κ chain/somatic mutation/transgene

Introduction

Somatic mutation of the V regions of antibody genes occurs during B cell development and contributes to the affinity maturation of the immune response (Berek and Milstein, 1987; Claflin *et al.*, 1987; Manser *et al.*, 1987; Rajewsky *et al.*, 1987; Blier and Bothwell, 1988). Mutations only accumulate several days after antigenic challenge and initial proliferation (Griffiths *et al.*, 1984) and their timing suggests that the germinal centres of spleen and lymph node are the likely sites of mutational activity (MacLennan and Gray, 1986). The molecular mechanism of hypermutation is not yet known, but it appears that a specific DNA targeting process is involved. Nucleotide changes are found only within a 2 kb zone around the rearranged V region (Gearhart and Bogenhagen, 1983; Both *et al.*, 1990; Lebeque and Gearhart, 1990), and mutations have been found on both productively and non-productively rearranged alleles from within the same hybridoma (Pech *et al.*, 1981; Roes *et al.*, 1989).

Ig transgenes introduced into the germline of mice can undergo somatic mutation. Nucleotide changes have been documented in a κ light chain transgene (O'Brien *et al.*, 1987; Hackett *et al.*, 1990) and may also have been found in an H chain transgene (Durdik *et al.*, 1989). In order to define the *cis*-acting DNA sequences required to target hypermutation to the antibody V region, we have compared transgene constructs based on a rearranged κ light chain gene containing the V_κOx1 V region which is specific for the hapten 2-phenyl-oxazolone (phOx). We have previously analysed a line of mice carrying a short version of this κ transgene which extends 1 kb 3′ of the C_κ gene (Sharpe *et al.*, 1990). We found that expression levels of this transgene are low and that the construct did not prevent rearrangement of the endogenous κ genes (i.e. it did not mediate allelic exclusion). Moreover, this construct was not a target for somatic hypermutation even in B cells in which the endogenous genes have been mutated. Following the identification of a second κ transcription enhancer element located 9 kb downstream of C_κ (Meyer and Neuberger, 1989), we made a second construct which extends 9 kb 3′ of C_κ. Transgenic mice now express this construct at levels similar to endogenous Ig genes, and the transgene causes allelic exclusion of endogenous κ in the majority of B cells (Meyer *et al.*, 1990).

In this paper, we show that the V region of the longer transgene construct is a substrate for somatic hypermutation. We find that the targeting and rate of mutation in antigen-selected B cells is comparable to that of the endogenous Ig genes even when the transgene κ chain does not form part of the expressed antibody molecule and is not therefore subject to direct selection.

Results

Production of phOx-specific hybridomas from transgenic mice

The transgene (Figure 1) encodes a κ light chain in which the V region is the mouse V_κOx1 which is the dominant light chain gene involved in the response to the hapten 2-phenyl oxazol-5-one (phOx); the C region is from the LOU strain of rat. This construct extends 9 kb downstream of the C_κ region and includes mouse κ intron and κ3′ enhancers (Meyer and Neuberger, 1989). Two independent transgenic lines were established which carried low copy numbers of integrated DNA (Meyer *et al.*, 1990). From Southern blot analysis of tail DNA, line $L\kappa^3$ carries ~3 copies, and $L\kappa^6$ ~5 copies (data not shown). Heterozygous mice back-crossed to BALB/c were immunized twice with phOx–chicken serum albumin (CSA) to generate a strong anti-phOx response (Griffiths *et al.*, 1984). Typical serum anti-phOx titres increased from <1/1000 pre-immune to 1/20 000 at 4 weeks after primary immunization (titres given

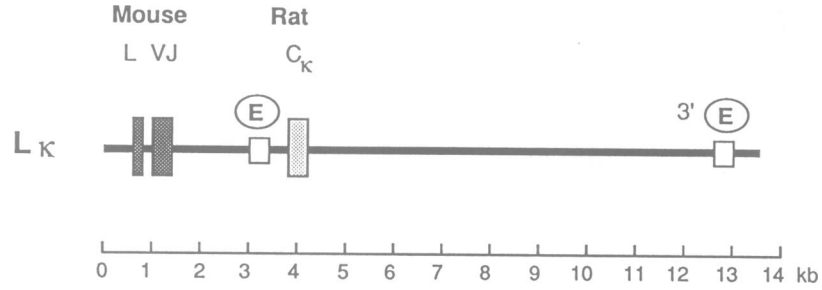

Fig. 1. The transgene κ construct. Coding region sequences are shown as large boxes, enhancers as small open boxes. Sequences of mouse origin are shown in heavy shading, and sequences of rat origin in light stippling.

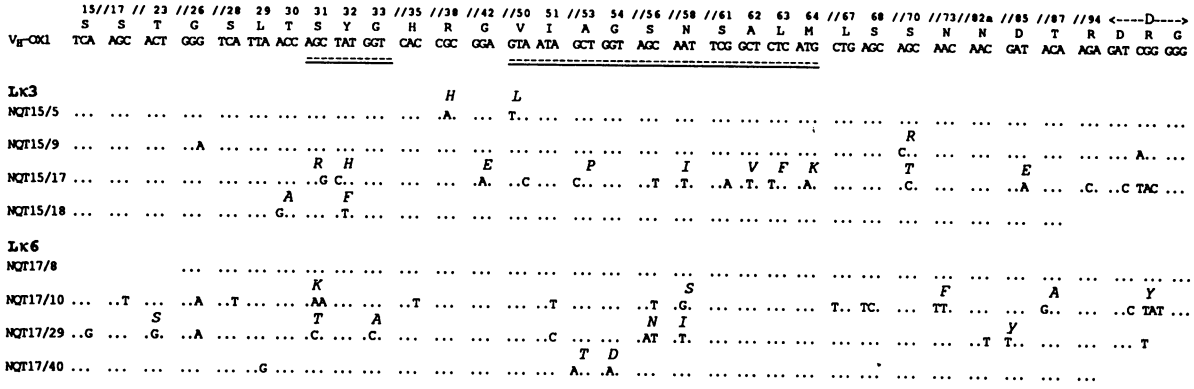

Fig. 2. Sequence mutations in the endogenous V_HOx1 in allelically-excluded anti-phOx hybridomas from transgenic lines $L\kappa^3$ and $L\kappa^6$. **Above:** the sequence of germline V_HOx1 (Kaartinen *et al.*, 1983) The amino acid translation is given, with numbering according to Kabat *et al.* (1987). Underlined codons represent residues in the CDR. **Below:** the sequences of four hybridomas from each transgenic line. Only those positions showing sequence differences from germline V_HOx1 are shown. A dot represents identity with the V_HOx1 sequence, a gap indicates that the nucleotide was not sequenced in this position. Predicted coding changes are indicated in italics.

as 50% binding to phOx−BSA). Hybridoma clones were generated from splenic B cells of mice immunized twice with phOx−CSA and culture supernatants were screened for transgene expression and phOx−BSA binding. Clones secreting IgG were selected since these are more likely to be derived from secondary response B cells. Hybridomas were then analysed by immunofluorescence for expression of transgene κ or endogenous mouse κ. The presence of the transgene prevented expression of an endogenous κ chain as the majority of the hybrids from lines and $L\kappa^3$ and $L\kappa^6$ expressed transgene κ only, although a small proportion in $L\kappa^3$ were identified which co-expressed both transgene and mouse κ.

The transgene V region undergoes somatic mutation

We initially analysed representatives of the major category of hybridomas, those in which the transgene is the only light chain expressed. Seven hybrids from $L\kappa^3$ (fusion NQT15) and seven from $L\kappa^6$ (fusion NQT17) were examined. In these, the endogenous H chain mRNA was cloned from four hybridomas of each line. All used the V_HOx1 gene, with a characteristic 3-residue D segment, encoding a V_H domain which is typically found in association with V_κOx1 (Kaartinen *et al.*, 1983). Most of these V regions show nucleotide changes from germline (Figure 2) confirming that somatic mutation had occurred in these B cells. There is a range of mutation from zero in NQT17/8 through two mutations in NQT15/9, to >11 mutations in NQT17/10, NQT17/29 and NQT15/17. However, it cannot be discounted that some of the latter sequence changes are encoded by genes closely related to V_HOx1 or by alleles inherited from the non-BALB/c background of the transgenic mice.

The sequences of the transgene κ chains were determined for all 14 hybridomas from cDNA clones generated by PCR amplification of specifically primed first strand cDNA. The sequences of the transgene V regions are shown in Figure 3. As the mice contain three ($L\kappa^3$) or five ($L\kappa^6$) copies of the transgene, at least 10 transgene cDNA clones were sequenced from each hybridoma. If a copy of the transgene has mutated, the same nucleotide change should be encountered in several independent cDNA clones. The cDNA clones can then be grouped according to mutation pattern. Nucleotide changes which result from errors inherent in the cloning steps occur at a frequency of $3-6\times10^{-4}$ (Sharpe *et al.*, 1990); these mutations are not repeated in independent clones, and have been omitted from the final analysis. It is clear from Figure 3 that the κ transgene is undergoing somatic mutation, to a degree roughly comparable to that of the endogenous H gene within the same cell. Each copy of the transgene mutates independently of the other copies within that cell and all transcribed copies can be mutated simultaneously (e.g. in hybridoma NQT17/45). Overall, the hybridomas from NQT17 ($L\kappa^6$) are more highly mutated than those from NQT15 ($L\kappa^3$) with the exception of hybridoma NQT15/17. Note that not all transcripts are necessarily represented for each hybridoma; either insufficient cDNA clones have been sequenced, or there were two unmutated copies which could not be distinguished from each other. In total, 94 transgene V region mutations were identified out of ~10 000 base pairs computed. However, 4 out of 14 hybridomas had no

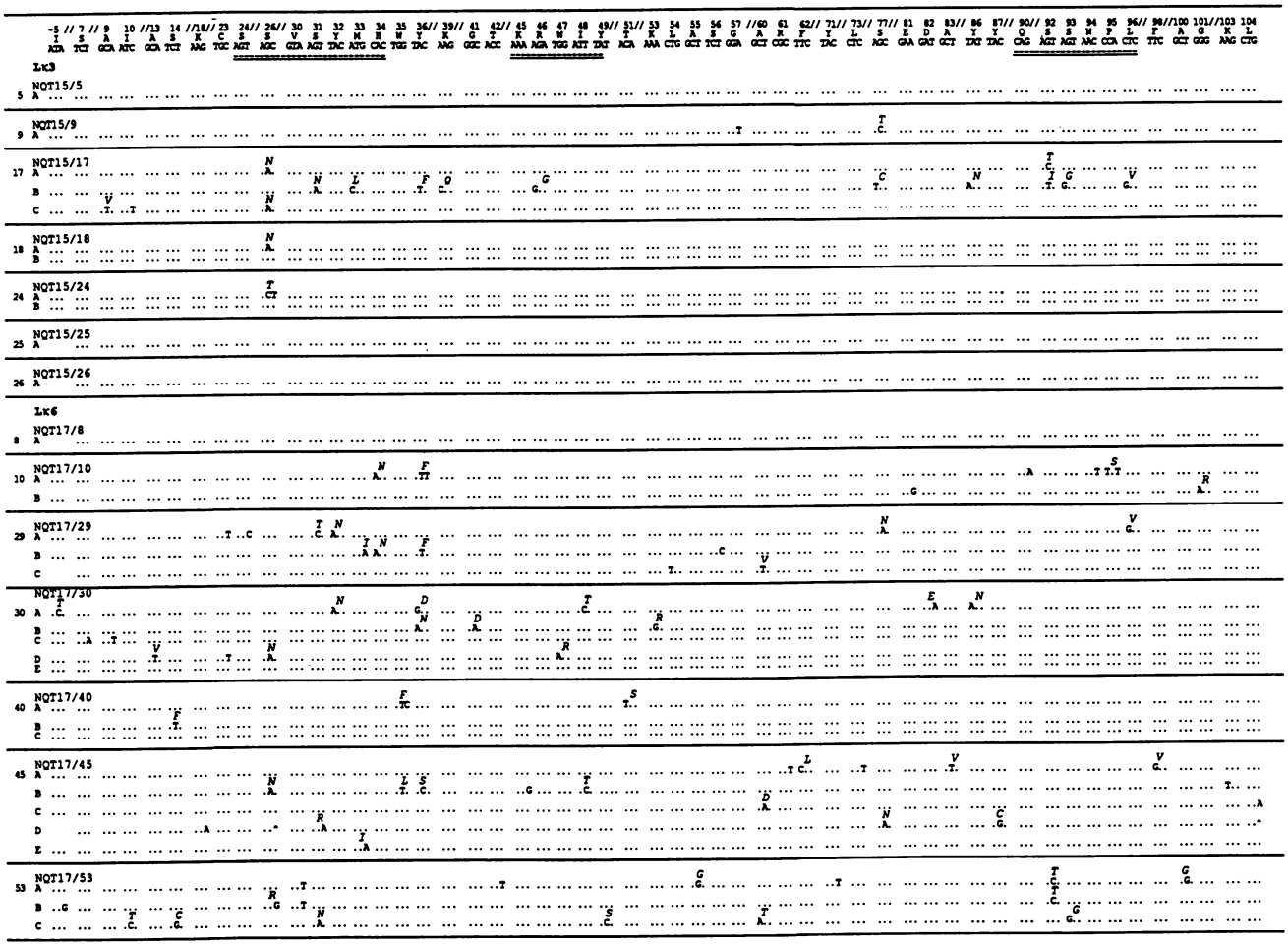

Fig. 3. Mutations in the transgene V regions in allelically excluded anti-phOx hybridomas The top line shows the sequence of the V_κOx1 transgene with amino acid translation above and numbering according to Kabat *et al.* (1987). Sequences of cDNA clones derived from seven hybridomas from each transgenic line are given below. Only the positions showing sequence differences from germline V_κOx1 are shown. For each hybridoma, groups of cDNA clones which represent an independently mutated copy of the transgene are indicated by A, B, C etc. (see text). The key for this figure is the same as for Figure 2. In addition, ^ represents positions that are probably identical to the germline sequence but whose identity could not be unambiguously established.

transgene mutations. Therefore, the average number of mutations found in hypermutated hybridomas was of the order of one mutation per 100 base pairs. In all cases, mutations are single base substitutions and no deletions or insertions were seen. Furthermore, the sequence of the C region (as well as that of the V region) was determined for all the mutated transgene cDNAs that were cloned. In all cases, mutations are correctly localized to the V region or leader sequence, and no sequence changes were found in the C domains in any of these transcripts (data not shown). These features indicate that the somatic hypermutation of the transgene parallels that of endogenous genes.

The V regions of passenger transgenes mutate

The presence of the transgene has not always prevented rearrangement and expression of an endogenous κ gene. This is particularly true in the Lκ^3 line, where 12 out of 27 anti-phOx hybridomas from fusion NQT15 co-expressed a mouse light chain with the transgenic κ. In eight of these twelve hybrids, we found that whilst RNA analysis and cytoplasmic immunofluorescence gave no reason to doubt that the transgene was well expressed, the transgene κ chains did not appear to be associated with the anti-phOx antibody as judged by ELISA of the culture supernatants (see below). We selected five of these eight hybridomas for further study. The following experiments show that the transgene κ does not contribute to the anti-phOx antibody in these hybrids. We compared two non-excluding hybrids which secreted the highest levels of transgenic κ (NQT15/4 and NQT15/23) with a typical hybridoma from the category that does not express an endogenous mouse κ gene (NQT15/18). Tissue culture supernatant was assayed for transgene κ content before and after passage through a protein A−Sepharose column. If transgene κ chain is associated with the endogenous IgG1, then the transgene will be retained on the protein A beads; free κ chains, however, will pass through the column. In hybridoma NQT15/18, most of the transgene activity is associated with the IgG1 H chain (Table I). In contrast, most of the small amount of transgene κ secreted by hybridomas NQT15/4 and NQT15/23 passed through the protein A column. The IgG-associated material was then eluted from the column and anti-phOx activity determined by binding to phOx−BSA coated plates. The plates were developed with reagents specific either for transgene κ or for total mouse Ig (Table I). As expected, the transgene κ is a component of the purified anti-phOx antibody from NQT15/18 whereas NQT15/4 and NQT15/23 antibodies contain no detectable transgene κ despite the high titre of

Table I. Comparison of binding characteristics of anti-phOx antibodies from allelically excluded and non-excluded transgenic hybridomas

Category	H chain (IgG1)	Mouse $\varkappa$	Hybridoma	Protein A–Sepharose column Transgene $\varkappa$ titre[a]		Binding of purified IgG to phOx-BSA	
				Pre-column	Post-column	Total Ig titre[b]	Transgene $\varkappa$ titre[c]
I. Excluded	V_HOx1	None	NQT15/18	$>10^4$	140	1000	750
II. Non-excluded	V_H11	$V_\varkappa$45	NQT15/23	170	160	45000	<0.2
			NQT15/4	35	30	5000	<0.2

For experimental details, see text. Titres are given as the dilution factor required to give 50% reaction in an ELISA.
[a]Indirect binding assay for transgene $\varkappa$ detected with antibody MARK-1; [b]Direct binding assay for total mouse antibody detected with sheep anti-mouse antiserum; [c]Direct binding assay for transgene $\varkappa$ detected with antibody MRC OX-12

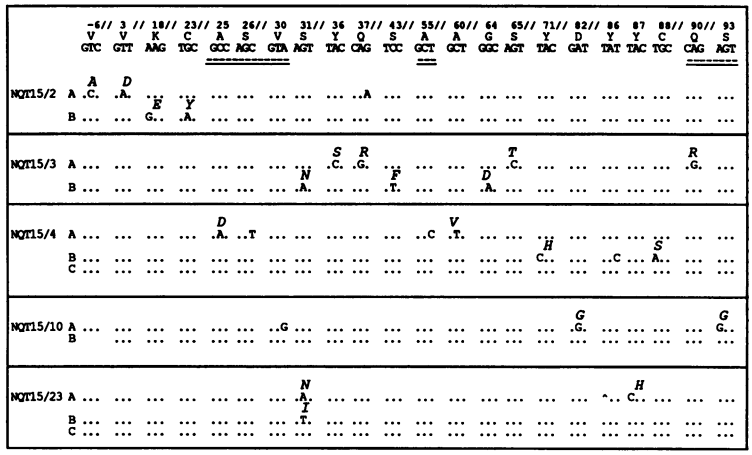

Fig. 4. Mutations in the $V_\varkappa$Ox1 transgene in the non-excluded anti-phOx hybridomas from L$\varkappa^3$ mice. Designations are as for Figures 2 and 3.

the mouse anti-phOx antibody in these preparations. Therefore, in the category of hybridomas co-expressing two $\varkappa$ chains, the transgene $\varkappa$ is secreted at a low level and hardly associates with the H chain and does not contribute to the antigen binding site. Thus the transgene can be considered a 'passenger' gene as selection pressures will act on the endogenous $\varkappa$ gene product.

The sequences of the passenger transgene $\varkappa$ were determined as before (Figure 4). Mutations are found in the transgene V region and are absent from the C region (data not shown). The extent of mutation is equal to or higher than that of the transgene in the previous (allelically excluded) category of B cells from line L$\varkappa^3$ (Figure 3) in which the transgene contributed to the antigen binding site. All copies of the transgene mutate independently. In this category of hybrids, three-quarters of the 25 mutations are transitions and one-quarter are transversions.

The sequences of the endogenous V_H and $V_\varkappa$ genes in the five hybridomas were determined as described before. In all five hybrids, the V_H gene is related to V_H11 (group 7) with a 5 amino acid long D segment, and the endogenous $\varkappa$ is related to $V_\varkappa$45.1 (Berek *et al.*, 1985). This gene combination is frequently encountered in the later stages of the immune response to phOx (Berek *et al.*, 1987). The $V_\varkappa$45.1 sequences contain a few somatic mutations (Figure 5), consistent with the observation that $V_\varkappa$45.1 is mutated only at a low frequency (Berek *et al.*, 1987). The V_H11 sequences (Figure 6) are very closely related to each other and all differ at eight positions from the germline sequence of V_H11 (Crews *et al.*, 1981). These nucleotide changes may be due to allelic differences or to the use of

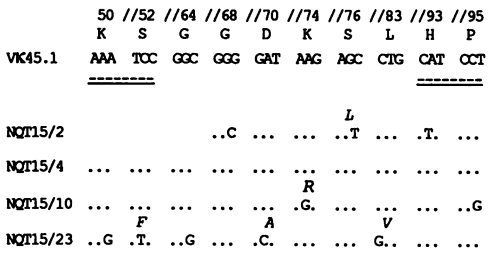

Fig. 5. Sequence mutations in endogenous $V_\varkappa$45.1 V regions in non-excluded anti-phOx hybridomas from line L$\varkappa^3$. The top line shows the sequence of the germline $V_\varkappa$45.1 (Berek *et al.*, 1985) with amino acid translation above and numbering according to Kabat *et al.* (1987). Sequences of four hybridomas are given below; only those positions showing sequence differences from germline $V_\varkappa$45.1 are indicated.

a different member of the V_H11 family due to the pedigree of these mice. Each heavy chain also contains unique changes which are likely to represent true somatic mutations.

Discussion

Somatic mutation may require sequences downstream of $C_\varkappa$

Our previous report (Sharpe *et al.*, 1990) that a $\varkappa$ transgene was not capable of somatic hypermutation contrasted with the studies on a different $\varkappa$ transgene by Storb and colleagues (O'Brien *et al.*, 1987; Hackett *et al.*, 1990). Several possibilities for this difference were discussed. In particular, the low level of expression of our transgene in the S$\varkappa$ lines leading to a lack of allelic exclusion was an important

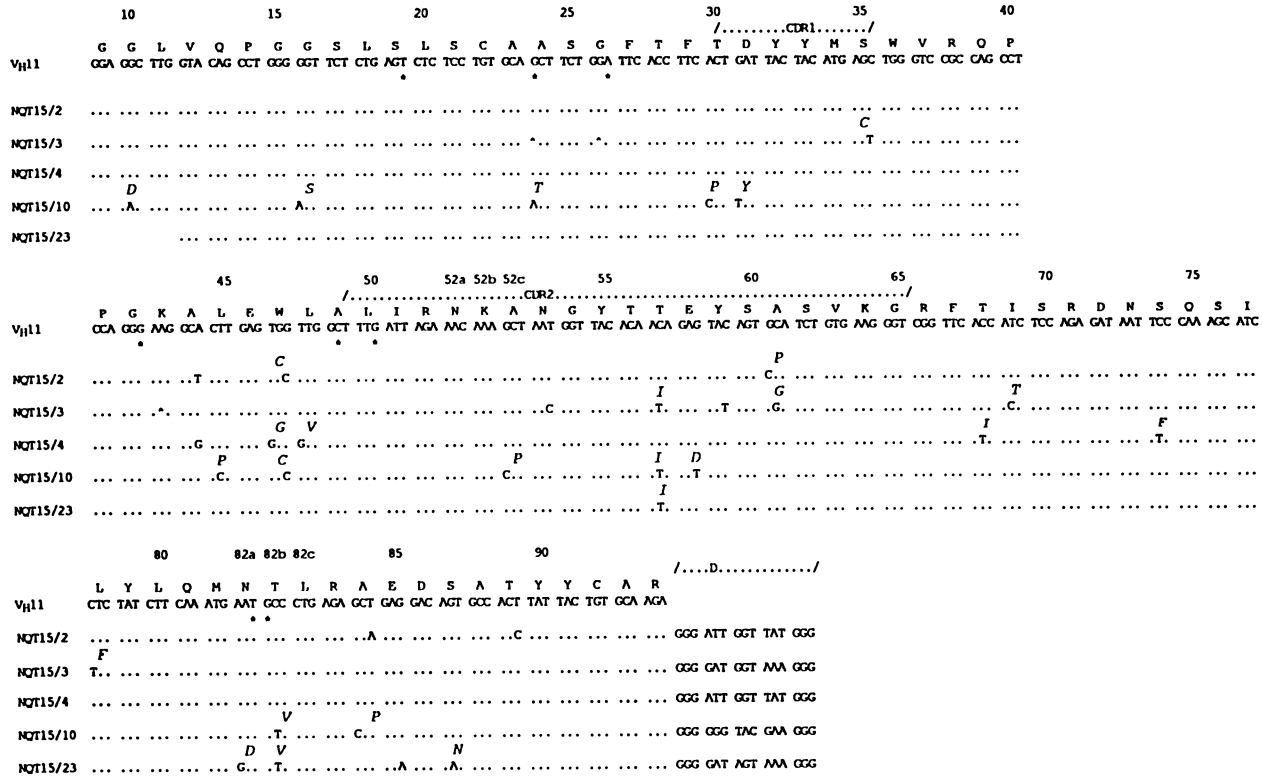

Fig. 6. Sequences of endogenous V_H regions of the non-excluded anti-phOx hybridomas from line $L\varkappa^3$. The top line shows the consensus sequence of the V_H11-like V region shared by all five hybridomas, with amino acid translation above and numbering according to Kabat *et al.* (1987) Residues which differ from V_H11 (Crews *et al.*, 1981) are indicated by an asterisk (*).

consideration. The demonstration that the recently described $\varkappa3'$ enhancer is necessary for good expression of $\varkappa$ transgenes (Meyer and Neuberger, 1989; Meyer *et al.*, 1990) suggested that this enhancer or other regions downstream of $C_\varkappa$ might also be necessary for somatic hypermutation. The present experiments were designed to explore such a possibility. As in the previous work, our new construct contains the $V_\varkappa Ox1$ gene (characteristically expressed in response to 2-phenyloxazolone) linked to rat $C_\varkappa$, but unlike the previous construct, the new one extends 9 kb (rather than 1 kb) downstream of $C_\varkappa$ so as to include the $\varkappa3'$-enhancer. We have studied two independent transgenic lines of mice, $L\varkappa^3$ and $L\varkappa^6$.

Practically all the anti-phOx hybridomas from the $L\varkappa^6$ line and 55% from $L\varkappa^3$ manifest allelic exclusion in that they only express the transgene and not an endogenously encoded $\varkappa$ chain. We find that the transgene V region undergoes somatic mutation. Individual transgene mutation patterns indicate that there are three transcripts in B cells from line $L\varkappa^3$ and five in line $L\varkappa^6$; this correlates with the estimated transgene copy number. Each actively transcribed copy of the gene is capable of becoming mutated, and each mutation pattern is independent of the others in the same cell. The nature and location of the mutations suggest that the transgene is being recognized by the same mechanism which targets the normal endogenous genes. The transgene mutations are correctly localized to the V region and are absent from the C region. Most importantly, this is true even when there are several gene copies in tandem head-to-tail arrangements. This shows that the mutation is targeted towards a specific gene segment. Within an individual anti-phOx B cell, the extent of mutation of the transgene roughly correlates with the extent of divergence of the V_H sequence from the sequence of V_HOx1. Thus, aside from *trans*-acting factors, the 13 kb $L\varkappa$ transgene construct contains all the DNA signals required for a normal level of correctly targeted somatic mutation.

If differences between the $S\varkappa$ and $L\varkappa$ transgenes in the J−C intron can be ignored (see Materials and methods), it is likely that an element downstream of $C_\varkappa$ is necessary in order to allow somatic mutation; this could conceivably be the $\varkappa3'$ enhancer itself. However, in the $L\varkappa^3$ line, we were able to derive some non-excluded anti-phOx hybridomas in which the transgene $\varkappa$ was secreted at a low level and had not prevented rearrangement of the endogenous $\varkappa$ alleles. In these $L\varkappa^3$ hybridomas, the transgene was somatically mutated even though it does not contribute to the antibody made by these cells. This finding suggests that, if it is the $\varkappa3'$ enhancer that is potentiating somatic hypermutation, then it is not having its effect by simply allowing the transgene to be expressed at a level sufficient for it to be selected by antigen. Moreover, Carmack *et al.* (1991) have found that a short $\varkappa$ transgene that lacks extensive 3′ sequence but which is nevertheless expressed at high level due to its integration position is not a substrate for somatic mutation. This suggests that the element(s) 3′ of $C_\varkappa$ which acts to potentiate hypermutation is not simply doing so by increasing the rate of transcription. Clearly, this element(s) needs to be defined more precisely and such experiments are in progress.

Mutations in the passenger transgene have not been selected by antigen

Mutations obtained on passenger transgenes may allow the study of hypermutation in the absence of selective pressure by antigen on the V region and provide information about

the intrinsic sequence preferences of the mutation machinery, such as mutational hotspots. In the hybridomas from the L$\varkappa^3$ line which co-express an endogenous mouse $\varkappa$ along with the transgenic $\varkappa$, the anti-phOx antibody is composed of an endogenously encoded $V_\varkappa$45.1 light chain associated with a V_H11-like H chain. The transgenic $\varkappa$ chain in these cells is secreted at a very low level and hardly associates with the H chain. Preferential pairing of cognate H and L chains is well documented (De Preval and Fougereau, 1976; Milstein and Cuello, 1983). If the H chain associates poorly with the transgenic $\varkappa$ chain, the cell would be expected to undergo rearrangement of one of its endogenous light chain loci and such cells may well have been specifically selected (for example, see Pettersson *et al.*, 1989). Furthermore, in cells expressing the high affinity $V_\varkappa$45.1/V_H11-like combination, the number of high affinity phOx binding sites expressed on the cell surface would not be affected by the presence of the transgene since the transgenic $\varkappa$ chain hardly associates with the H chain.

Since mutations on passenger transgenes occur at a similar rate to those on selectable genes, selection seems to have only a marginal effect on the frequency of accumulated mutants. This is not surprising as the rate of mutation is so much higher than the rate of fixation of mutants by antigen selection (Berek and Milstein, 1988). This also agrees with reports that non-productively rearranged alleles mutate at similar frequencies to productively rearranged alleles (Pech *et al.*, 1981; Roes *et al.*, 1989).

The pattern of transgene hypermutation

The mutations in both categories of transgenic hybridomas are single base changes—no deletions or insertions are found. This is similar to the observation for endogenous V region genes, although Lebeque and Gearhart (1990) have observed some deletions/insertions in 5′ and 3′ flanking regions.

It is obviously of interest to compare the detailed pattern of mutation observed in the transgenic $\varkappa$ chains with what has been found in phOx-specific hybridomas from non-transgenic mice. Certain mutations, in particular His34→Asn and Tyr36→Phe, are typically associated with affinity maturation to phOx and are observed in about three-quarters of secondary response $V_\varkappa$Ox1 genes in hybridomas from BALB/c mice (Berek and Milstein, 1987). We do see these same two mutations in two of the transgenic hybridomas which belong to the category of hybrids (category I) in which the antibody light chain is encoded by the transgene. We do not see these mutations in category II hybridomas in which the transgene is a passenger. Therefore, it is clear that the transgene can be mutated and selected in a manner similar to endogenous $\varkappa$ genes. Furthermore, it indicates that the His34 and Tyr36 mutations are selected by antigen and their occurrence does not simply reflect that they are mutational hotspots.

However, it is clear that the His34→Asn and Tyr36→Phe mutations are not as commonly observed amongst the category I transgenic hybridomas as they are amongst phOx-specific hybridomas from BALB/c. We believe that a likely explanation for this is that in the category I hybridomas, only one of the transgene copies is mutated such that it expresses a $\varkappa$ chain which yields a higher affinity phOx binding site; the other transgene copies within the same cell are likely to act as competitors and not as simple passengers. Indeed, support for this proposal is found in the observation that some of the category I transgenes have mutations which probably disrupt the structure of the light chain (e.g. mutation of Trp35 in NQT17/40A and NQT17/45B or of Pro95 in NQT17/10A) or destroy or impair contacts that have been established from X-ray diffraction studies (Alzari *et al.*, 1990) as being important in antigen binding (e.g. mutation of Tyr32 to Asn in NQT17/29A and NQT17/30A or mutation of Tyr36 to Ser in NQT17/45B or to Asp in NQT17/30A). Such deleterious mutations could have been selected as they might prevent association with the H chain and thus allow all the expressed antibody to be derived from the other mutated transgene copy within the cell which confers higher affinity phOx binding.

Finally, we come to the question of mutational hotspots. The results obtained here have already demonstrated that some of the hotspots characteristic of secondary anti-phOx antibodies (Tyr34 and His36) must occur largely in response to antigenic selection. Analysis of the mutation of the transgene reveals additional hotspots—mutation of Ser26 is seen repeatedly in the transgene but has never previously been observed as a replacement mutation in secondary anti-phOx hybridomas. However, it is one of the few examples of a position which repeatedly exhibits silent changes (Berek and Milstein, 1987). Furthermore, the transgene copies which harbour mutations of Ser26 are not the ones which also harbour changes associated with increased affinity for phOx. Therefore, Ser26 could well be an intrinsic mutational hotspot which is not detected in the usual secondary anti-phOx response because alterations of this residue could be harmful for antigen binding. However, an alternative explanation which we cannot yet rule out is that, as discussed above, deleterious mutations of some of the transgene copies could even confer a selective advantage and Ser26 mutations could constitute just such an example. We intend to avoid this sort of ambiguity in future by analysing a $\varkappa$ transgene that cannot be translated.

In contrast to Ser26, the Ser31 codon constitutes a mutational hotspot where this uncertainty does not apply. Mutations of Ser31 are seen in about a quarter of secondary response anti-phOx hybrids of BALB/c mice; mutations of this residue are also common in both the category I and the category II hybrids from the transgenic mice. It is therefore clear that this residue is an intrinsic mutational hotspot. Other examples [e.g. Tyr36 (in addition to its being a target for antigen selection) as well as Ala60] exist but the case is at present less convincing. Clearly, it will be of interest to identify further examples of mutational hotspots and such information should come out from a more extensive analysis of mutations in passenger transgenes.

Materials and methods

Transgene construct and transgenic mice

The construction of the L$\varkappa$ transgene (Figure 1) has been described previously (Meyer *et al.*, 1990). It is an extended version of the S$\varkappa$ construct (Pettersson *et al.*, 1989) and differs from S$\varkappa$ in that the $J_\varkappa - C_\varkappa$ intron is now derived from mouse DNA, and there is an additional 8 kb of DNA 3′ of the $C_\varkappa$ gene. Transgenic mouse lines L$\varkappa^3$ and L$\varkappa^6$ carrying the L$\varkappa$ construct have been described previously (Meyer *et al.*, 1990). Transgenic mice were produced by microinjection into (C57BL/6×CBA)×(C57BL/6×CBA) eggs and founder mice crossed to BALB/c for two generations.

Immunization and derivation of anti-phOx hybridomas

L$\varkappa$ mice were immunized as described previously (Griffiths *et al.*, 1984) with 100 μg phOx-CSA i.p., then boosted 5.5 weeks later (L$\varkappa^3$) or 7 weeks

later ($L\kappa^6$) with 100 μg soluble phOx-CSA i.v. After 3 days, splenic B cells were fused with NSO (Galfré and Milstein, 1981) and hybridomas cloned in soft agar.

Serological assays and immunofluorescence

Transgene κ chains were detected by an ELISA using the mouse anti-rat monoclonal antibody MARK-1 as previously described (Pettersson *et al.*, 1989, Sharpe *et al.*, 1990). In some assays, two modifications were made: (i) plates were coated with 5 μg/ml of the rat κ light chain Y3 (Cotton and Milstein, 1973) and (ii) the assay was developed with avidin–horseradish peroxidase (Dako, High Wycombe, UK). Antibodies specific to phOx were assayed by binding to phOx–BSA coated plates (Sharpe *et al.*, 1990). Transgene κ chains were detected with biotinylated MRC OX12 1/1200 (Serotec, Kidlington, UK) and total mouse Ig was detected with horseradish peroxidase conjugated rabbit-anti-mouse (Dako). H chain subclass was determined using a mouse monoclonal typing kit (Serotec).

Immunofluorescence staining of permeabilized hybridoma cells was performed as previously described (Pettersson *et al.*, 1989). Mouse κ was detected with FITC OX-20 and transgene κ with biotinylated sheep anti-rat antiserum in the presence of 1% mouse serum followed by TRITC–streptavidin (Serotec).

Protein A binding

Hybridoma supernatant (1.6 ml) was passed through a 1 ml protein A–Sepharose CL 4B column (Pharmacia) (Harlow and Lane, 1988). Material which passed through the column was collected and assayed for trangene κ.

Ig cDNA cloning

Transgene and endogenous Ig gene transcripts were cloned from specifically primed first strand cDNA by PCR amplification using the primers and protocol described before (Sharpe *et al.*, 1990). PCR products were digested with restriction enzymes, cloned into M13 and sequenced with Sequenase (USB, Cleveland, Ohio). Minor amendments to the cloning protocol were as follows: transgene cDNA was amplified for 12–14 cycles, of 94°C for 1.5 mins, 55°C for 2 min and 72°C for 2 min. Endogenous V cDNAs were amplified for 30 cycles using the back primer $V_\kappa$back (Orlandi *et al.*, 1989) and V_κ45 V regions sequenced with the primer V_κ45 (Kaartinen *et al.*, 1983).

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