Nucleotide sequence and properties of the murine γ_3 immunoglobulin heavy chain gene switch region: implications for successive C_γ gene switching

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Abstract

During B lymphocyte differentiation, immunoglobulin heavy chain constant region (C_H) genes undergo a unique series of DNA recombination events culminating in the C_H class switch. C_H switch (S) regions are located 2 kb 5' of each C_H gene except δ (i.e. μ , γ_3 , γ_1 , γ_{2b} , γ_{2a} , ϵ and α). We describe the structural features of the γ_3 switch region. Hybridization experiments show that S_{γ_3} has remarkable homology to both S_μ and other S_γ regions while S_μ possesses limited homology to the other S_γ sequences. However, S_μ possesses extensive sequence homology with S_ϵ and S_α . The nucleotide sequence of S_{γ_3} reveals higher densities of S_μ repetitive sequences (GAGCT and GGGGT) and another S region common sequence (YAGGTG) than observed for S_{γ_1} , $S_{\gamma_{2b}}$ or $S_{\gamma_{2a}}$. In addition, the conservation of S_μ like repetitive sequences in S_γ regions is correlated with the 5'→3' γ gene order (i.e. $S_{\gamma_3} > S_{\gamma_1} > S_{\gamma_{2b}} > S_{\gamma_{2a}}$). A model is presented which suggests that the unique features of S_{γ_3} may allow for successive switches from C_μ to any C_γ gene.

INTRODUCTION

Switches in the expression of immunoglobulin heavy chain constant regions (C_H) occur during B lymphocyte differentiation (1-3). A unique series of DNA recombination events which result in the deletion of C_H genes have been implicated in the switching process (4-9). C_H gene deletions that are correlated with C_H switching have been characterized in mouse plasmacytomas (4-9), hybridomas (10), tissue culture variant myeloma cell lines (11) and lymphomas (12). C_H gene deletion events have been utilized to determine the overall organization of the C_H gene cluster which has subsequently been confirmed by molecular cloning (i.e. μ - δ - γ_3 - γ_1 - γ_{2b} - γ_{2a} - ϵ - α) (13). C_H gene deletions associated with switch-recombination have been proposed to occur by either intrachromosomal deletions (4,9) or by sister chromatid exchanges (14).

Two models have been proposed for the molecular mechanism(s) of C_H switch-recombination (15,16). The first suggests that C_H class specific

DNA recombinases recognize intrinsically different repetitive DNA sequences localized within intervening DNA sequences 5' of each C_H gene (S regions) (15). The second model does not preclude the basic tenants of the first but instead proposes that two short sequences (i.e. GAGCT and GGGGT) common to all S regions mediate C_H switching by homologous DNA recombination (16). The second model is supported by the fact that a uniquely large GAGCTG repeat within S_μ and a significant portion of S_α are both conserved in mice and humans (17-19). In addition to these putative switch-recombinase recognition sequences, we have recently demonstrated that analogues of a YAGGTG prototype sequence are found immediately 5' of the recombination sites of functionally rearranged C_H genes and are also repeated in S regions (20,21).

In this report, we present the nucleotide sequence of the S_{γ_3} region and describe its hybridization properties with other S regions. In consideration of these results and other published cellular data on successive C_H gene switching (11,22-25), we present a model suggesting that under the appropriate circumstances the S_{γ_3} region may allow for successive C_H gene switches.

MATERIALS AND METHODS

Molecular cloning and Southern blot hybridizations (26) were all performed as described in previous publications (20,27). The S_{γ_3} region was subcloned into pBR322 and sequenced according to Maxam and Gilbert (28).

RESULTS

We have recently obtained a γ_3 genomic clone by screening a Balb/c sperm library with a $C_{\gamma_{2b}}$ cDNA probe (20). A restriction map of this clone, SL51, was derived as shown in Fig. 1. SL51 was shown to contain the C_{γ_3} gene by comparison to other published data (29) and by cross-hybridization with a $C_{\gamma_{2a}}$ cDNA clone (30). To determine if SL51 contained S_{γ_3} sequences, Southern hybridization analyses (26) of restriction enzyme digests of SL51 were performed using a variety of heavy chain switch region DNA probes. As shown in Figures 2C (lane 4) and 3A (lane 2 and 2'), strong hybridization of a 0.8 kbp RI-HindIII fragment of SL51 to both our $S_{\gamma_{2b}}$ and S_μ probes (PBR1.4 and pM2-20 respectively in Fig. 1) indicates that the S_{γ_3} region lies about 1.9 kbp 5' of the γ_3 coding region (see SL51 clone map in Fig. 1). This result

ORIGIN OF C_H GENE 5' FLANKING DNA SEQUENCE PROBES AND LOCATIONS OF C_H GENE SWITCH SITES

Figure 1.

Restriction maps of seven heavy chain constant region genomic clones and their 5' and 3' flanking sequence. Plasmid subclones of C_H switch regions are indicated above and below the restriction maps. The DNA sequencing strategies for S_{γ_3} are shown below the γ_3 genomic clones SL51 + SL14. M23 (27), SL19 δ (33, this paper) IgC γ_1 (36), SL1 γ_{2b} (20), γ_{2a-9} (31), M64 + M67 (27), SL51 + SL14 (20, this paper).

would appear to be in good agreement with other known switch regions which are also shown in Figure 1.

While determining the location of the S_{γ_3} region in SL51, we observed that our S_μ probe exhibited more homology to S_{γ_3} than to our

S_{Y2b} containing genomic clones, SL59 and SL1 (20). To facilitate our analysis of S_{Y3} , a 2.7 kbp RI-BamHI fragment containing the S_{Y3} region and 1.9 kb of 3' flanking sequences was prepared for a hybridization probe (see pS_{Y3} 2.7 in Fig. 1). Using an S_{μ} probe ($pM2-20$), Southern blot hybridizations were performed with the purified 2.7 kbp S_{Y3} containing restriction fragment, the original γ_3 genomic clone (SL51), a γ_{2b} genomic clone (SL1) that contains the entire S_{Y2b} region and the 5' portion of S_{Y2a} (20) and a restriction fragment spanning the entire S_{Y2b} region. The results of this experiment are shown in Figure 3A and the clones used are depicted in Figure 1. To allow for a more thorough comparison of the quantitative difference in S region homologies, 1X and 10X exposure times of this experiment are shown in Figure 3A. We have also investigated the homology of the entire S_{Y2a} region to S_{μ} by hybridizing an S_{μ} probe to a genomic clone containing the complete $\gamma_{2b}-\gamma_{2a}$ intragenic region ($\gamma_{2a}-9$ in Figure 1) (data not shown). These results clearly demonstrate that S_{μ} possesses considerably more S_{Y3} homology than S_{Y2b} or S_{Y2a} homology. Cross-hybridization experiments between S_{μ} and S_{α} indicate a high degree of homology (see Figure 2A) which appears comparable to the level of $S_{\mu}-S_{Y3}$ homology.

The relative homology of S_{Y3} with other S regions was determined with an S_{Y3} probe (S_{Y3} 2.7 in Fig. 1). As shown in Figure 3B (lanes

Figure 2.

A. Cross-hybridization of S_{α} and S_{μ} sequences with a complete S_{α} probe ($p\alpha 64-101$). Track #1: HindIII digestion of μ clone M2, Track #2: EcoRI digestion of μ clone M2, Track #3: HindIII digestion of α clone M64 and Track #4: Molecular weight marker mix containing a 14.5 kb EcoRI fragment of a J558 rearranged α gene (37). Weakly hybridizing bands in Track #3 are due to deleted forms of the S_{α} region (32).

B. Cross-hybridization of S_{μ} and S_{α} sequences with an S_{μ} probe ($pM2-20$). Track #1: EcoRI digestion of α clone M64, Track #2: Molecular weight marker mix, Track #3: HindIII digestion of μ clone M2 and Track #4: EcoRI digestion of μ clone M2.

C. Cross-hybridizations of S_{Y2b} and S_{Y3} sequences with an S_{Y2b} probe ($pBR1.4$). Track #1: EcoRI digestion of γ_3 clone SL51, Track #2: EcoRI+BamHI double digestion of SL51, Track #3: Molecular weight marker mix, Track #4: EcoRI+HindIII double digestion of SL51 and Track #5: KpnI digestion of SL51.

All experiments were performed on 0.8% agarose gels which were transferred to nitrocellulose filters and hybridized as previously described (20,27). Ethidium stained gels are shown adjacent to the appropriate autoradiographs for comparison. Hybridizing bands are identified to the right of the ethidium pictures by their gel track numbers. Equivalent weights of DNA samples (following corrections for sequence length) were applied to all tracks.

3,4,6-8), the S_{Y3} region possesses extensive homology with S_{Y1} , S_{Y2a} and all the repeating units that define S_{Y2b} . A HindIII digestion of a 10.2 kb RI fragment containing the C_{μ} gene and neighboring sequences (see clone M2 in Fig. 1) contains only one S_{Y3} homologous band corresponding to the S_{μ} region (lanes 10 and 10' of Fig. 3B). Therefore, the S_{Y3} 2.7 probe does not possess detectable sequence homology to DNA sequences 3' of C_{μ} which span the μ - δ intragenic region (33) (see Fig. 1 for restriction maps). S_{μ} and S_{α} DNA probes also fail to hybridize to restriction fragments corresponding to the μ - δ intragenic region (see Fig. 2A (13)). These findings would collectively argue that S_{Y3} uniquely possesses a high density of both S_{μ} and S_{γ} like sequences while DNA sequences 5' of C_{δ} possess no detectable S region homology. A summary of these results is present in Figure 4.

To further investigate the novel features of S_{Y3} , we determined the complete nucleotide sequence of the S_{Y3} region in our S_{Y3} 2.7 probe. An 800 bp RI- H_3 fragment that contains S_{Y3} was subcloned into pBR322, and DNA sequencing strategies are shown in Figure 1. The complete sequence

Figure 3

A. Comparative cross-hybridization of S_{Y3} , S_{Y2b} and S_{Y2a} sequences with an S_{μ} probe (pM2-20). Track #1: RI-BamHI insert of p S_{Y3} 2.7, Track #2: RI + HindIII double digestion of p S_{Y3} 2.7 insert, Track #3: RI + BamHI double digestion of SL51, Track #4: molecular weight marker mix, Track #5: EcoRI digestion of clone SL59 Y_{2b} , Track #6: EcoRI digestion of clone SL1 Y_{2b} , Track #7: KpnI digestion of clone SL1, Track #8: 3.9 kb S_{Y2b} restriction fragment of clone SL1, Track #9: KpnI digestion of Track #8 DNA.

B. Comparative cross-hybridization of S_{Y3} , S_{Y1} , S_{Y2b} , S_{Y2a} , S_{α} and S_{μ} with an S_{Y3} probe (p S_{Y3} 2.7). Track #1 and #2: p S_{Y3} 2.7 insert, Track #3: Ig C_{Y1} insert, Track #4: KpnI digestion of clone SL1, Track #5: Molecular weight marker mix, Track #6: 3.9 kb S_{Y2b} restriction fragment of clone SL1, Track #7: KpnI digestion of Track 6 DNA, Track #8: BglII digestion of Track 6 DNA, Track #9: 2.0 kb EcoRI-SacI DNA fragment from clone SL1 Y_{2b} (containing 1.4 kb of C_{Y2b} 5' flanking DNA, C_{H1} and Hinge sequences of C_{Y2b} as shown in Fig. 1), Track #10: pM2-20 insert, Track #11: SacI digestion of the pM2-20 insert, Track #12: HindIII digestion of μ clone M2.

Similar quantities of DNA fragments with appropriate length corrections are applied to all tracks. Tracks 1'-9' in Panel A and Tracks 1'-12' in Panel B represent increased exposures obtained with intensifier screens. Ethidium pictures of the appropriate agarose gels are shown for comparison. Hybridizing bands corresponding to various C_{μ} switch regions (see Fig. 1 for restriction maps and switch region locations) are indicated to the right of each autoradiograph and ethidium picture with their appropriate track numbers. A weak band in track 10' indicated by an asterisk (*) represents an S_{μ} partial digestion product. An S_{μ} partial digestion band is also present in lane 12'.

COMPARATIVE DNA SEQUENCE HOMOLOGIES
OF MOUSE C_H GENE SWITCH REGIONS

SWITCH REGION RESTRICTION FRAGMENTS	HYBRIDIZATION PROBES							
	S_μ	$5'C_8$	S_{γ_3}	S_{γ_1}	$S_{\gamma_{2b}}$	$S_{\gamma_{2a}}$	S_α	
	S_μ	++	ND	+	ND	+-	ND	+
	$5'C_8$	-	++	-	ND	-	ND	-
	S_{γ_3}	+	ND	++	ND	+	ND	+
	S_{γ_1}	+-	ND	+	++	+	ND	-
	$S_{\gamma_{2b}}$	+-	ND	+	ND	++	ND	-
	$S_{\gamma_{2a}}$	+-	ND	+	ND	+	++	-
	S_α	+	ND	+	-	-	ND	++

++ = IDENTICAL SEQUENCES
+ = SUBSTANTIAL SEQUENCE HOMOLOGY
+- = LOW BUT DETECTABLE SEQUENCE HOMOLOGY
- = NEGLIGIBLE OR NO SEQUENCE HOMOLOGY
ND = NOT DETERMINED

Figure 4.

A qualitative summary of the data presented in Figures 2 and 3.

of the insert of pS_{γ_3} 0.8 is presented in Figure 5. The γ_3 gene switch region is composed of a repeating unit of about 49 bp. This is analogous to other γ switch regions where 49 bp repeats have also been described (34). The S_{γ_3} repeating units would appear to be more highly conserved than the repeats within other S_γ regions (34). A prevalent sequence for the S_{γ_3} repeating unit was deduced from the nucleotide sequence. A comparison of the S_{γ_3} prevalent sequence to the consensus sequences of other heavy chain switch regions is presented in Figure 6. The homologies exhibited between S_{γ_3} and the other S_γ regions detected by hybridization experiments in Figures 2C and 3B are easily explained. All S_γ regions exhibit a relatively high degree of sequence homology. In addition, the degree of sequence homology shared by the four S_γ regions would appear to reflect the chromosome order of the γ genes

S REGION
PREVALENT
SEQUENCES:RELATIVE
HOMOLOGIES (MAX)

$S_{\gamma 3}$	TPTGGGGACCAGGCTGGGCAGCTCYPGGGGAGCTGGGGTAGGTGGGAP	100		
$S_{\gamma 1}$	TPTPGGP ^T CCAGGCTGAGCAGCTACAGGGGAGCTGGGGYAPPTGGGAP	95	100	
$S_{\gamma 2b}$	TPTGGGGACCAG ^A TCTAGCAGCTPTGGGGGAGCTGGGGA ^A GGTTPGGAP	87	88	100
$S_{\gamma 2a}$	NGTGGGGACCAGGCAGTACAGCTCTGGG ^T PGGGNCAGG-CAG-TACAGG ^{CTCT}	70	70	70
S_{ϵ}	GGGCTGGGCTGAGCTGPGCTGAGCTGPGCTGAGCTGPPNT			
S_{α}	ATGAGCTGGGATGAGCTGAGCTAGGCTGGAATAGGCTGGGCTGGTGTGGAGCTGGGTAGGCTGAGCTGAGCTGGA			
S_{μ}	GAGCTGAGCTGGGGTGAAGCT			

Figure 6

Comparisons of structural features of prevalent repeat sequences of $S_{\gamma 3}$ (this paper), $S_{\gamma 1}$ (34), $S_{\gamma 2b}$ (34), $S_{\gamma 2a}$ (35), S_{α} (14,15), S_{μ} (16) and S_{ϵ} (35). S_{γ} sequence homologies were determined on the basis of a 48 mer consensus repeat length and are indicated to the right of each S_{γ} prevalent sequence. Gaps and insertions were introduced to yield the maximum length of homologous DNA sequences. S_{γ} homology to S_{ϵ} , S_{α} and S_{μ} were not determined due to their radically different repeat lengths.

(i.e. $S_{\gamma 3} > S_{\gamma 1} > S_{\gamma 2b} > S_{\gamma 2a}$). However, the high degree of sequence homology between the γ switch regions does not help to explain why $S_{\gamma 3}$ uniquely displays strong homology to S_{μ} while the other γ switch regions only weakly hybridize to S_{μ} . The $S_{\gamma 3}$ sequence contains 35% more of the S_{μ} repetitive sequences, GAGCT and GGGGT, than an analogous length of $S_{\gamma 2b}$ (34), and this is reflected to some degree in the $S_{\gamma 3}$ prevalent sequence (see Fig. 6). The reason for the high S_{μ} - $S_{\gamma 3}$ homology exhibited in the hybridization experiments is also evident from the fact that two of the $S_{\gamma 3}$ 49 bp units contain an internal tandem repeat which closely resembles the sequence organization of the S_{μ} tandem repeat (16). $S_{\gamma 3}$ also possesses the short sequence CAGCTC in most of its repeating units. This sequence is the complement of GAGCTG, the highly repeated 6 mer found within the μ switch region. These results indicate that both DNA strands of the $S_{\gamma 3}$ region have sequence homology to the S_{μ} repeats.

DISCUSSION

We have presented the nucleotide sequence of the 3' end (~900 bp) of the Balb/c $S_{\gamma 3}$ region. Using these results and data obtained by others, the entire length of $S_{\gamma 3}$ is estimated to be ~4 kbp which is

comparable to the size of $S_{\gamma 2b}$ (34). However, the 49 bp $S_{\gamma 3}$ repeat unit would appear to be poorly conserved at the extreme 5' and 3' ends of the $S_{\gamma 3}$ region (see Fig. 5) (34, this paper). The switch-recombination site of the expressed γ_3 gene of the J606 Balb/c myeloma has recently been determined and is located ~300 bp 5' of the $S_{\gamma 3}$ sequence reported here (35). The $S_{\gamma 3}$ 49 bp repeating unit near the J606 γ_3 gene recombination site is virtually identical in nucleotide sequence characteristics to the ~900 bp of $S_{\gamma 3}$ sequence presented in this report.

Hybridization and DNA sequence data presented here show that the γ_3 switch region possesses substantially more S_{μ} homology than observed with other S_{γ} regions. $S_{\gamma 3}$ contains a high density of the S_{μ} repetitive sequences, GAGCT and GGGGT. Examples of YAGGTG like sequences, which have been implicated in C_H switch-recombination (20,21), are more prevalent in $S_{\gamma 3}$ than in other S_{γ} regions (see Fig. 6). The degree of divergence of S_{μ} like sequences within the different S_{γ} regions appears to follow the C_{γ} gene order (i.e. $S_{\gamma 3} < S_{\gamma 1} < S_{\gamma 2b} < S_{\gamma 2a}$) (see Fig. 6). However, S_{ϵ} and S_{α} possess the highest degree of S_{μ} homology (13,35, this paper) even though the ϵ and α genes follow γ_{2a} in the C_H gene order (13,31).

Implications for Successive C_{γ} Gene Switching

When a switch from C_{μ} to a C_{γ} gene occurs during an immune response, an initial encounter with $S_{\gamma 3}$ may help to facilitate further switching to other C_{γ} genes. The strong homology between S_{μ} and $S_{\gamma 3}$ and the high density of other switch site recognition sequences in $S_{\gamma 3}$ (i.e. YAGGTG like sequences) could conceivably favor such a phenomenon. At this point, either the γ_3 gene would be expressed or a transient S_{μ} - $S_{\gamma 3}$ recombination event would allow switching to continue to downstream genes. The homologies displayed between $S_{\gamma 3}$ and the other S_{γ} regions which are not shared by S_{μ} would accommodate this subsequent event. In addition, we would also predict that C_{ϵ} and C_{α} switches would not require $S_{\gamma 3}$ mediation since S_{ϵ} and S_{α} already possesses extensive homology with S_{μ} (13,32,35). This model would require an initial recombination event between the S_{μ} GAGCT tandem repeat (16) and the homologous $S_{\gamma 3}$ region. We have recently shown that the S_{μ} sites employed by nine rearranged C_H genes (representing γ_1 , γ_{2b} , and α isotypes) are located 3' of a YAGGTG consensus sequence and not within the S_{μ} GAGCT repeat (21). GAGCT tandem repeats do not appear to directly participate in most C_H gene switches and appear to be deleted

in the initial recombination event (21). However, the S_{μ} site employed by the J606 γ_3 gene is found within S_{μ} tandem GAGCT sequences (35).

Myeloma and hybridoma cell lines are known to possess the potential to undergo successive C_H gene switches *in vitro*. The MPC-11 myeloma is capable of generating $\gamma_{2b} \rightarrow \gamma_{2a}$ switch variants (22) by a DNA rearrangement which deletes the parental cell's expressed γ_{2b} gene (11). The X-63 hybridoma cell line undergoes spontaneous successive C_H switches (i.e. $\gamma_3 \rightarrow \gamma_1 \rightarrow \gamma_{2b} \rightarrow \gamma_{2a}$) at low frequency (i.e. 10^{-6} - 10^{-7} per cell per generation) which are correlated with C_H gene rearrangements (23,24). The role of a successive C_H gene switching pathway in normal B cell isotype switches remains to be determined.

Recent observations by Mongini, Paul and Metcalf on the C_H switching patterns of normal B cell clones responding to TNP ficoll (a T cell independent, Type II antigen) would be compatible with a successive C_H gene switching mechanism (25). In this study, B cell clones reacting with TNP-ficoll were prepared by the splenic-focus procedure in irradiated nude mouse recipients (25). All cultured B cell clones reacting with TNP-ficoll were found to express C_{μ} . However, the majority of B cell clones which also co-expressed a γ isotype other than γ_3 invariably expressed γ_3 as well. These results in combination with the unique structural features of S_{γ_3} defined in this report would collectively argue that switches to C_{γ} genes may proceed through an initial $S_{\mu} \rightarrow S_{\gamma_3}$ event in some instances. An initial $S_{\mu} \rightarrow S_{\gamma_3}$ event may be preferred in certain immune responses (i.e. T cell Independent responses) but need not be an obligatory prerequisite for all $\mu \rightarrow \gamma$ switches. Interestingly, B cell clones which produce IgA and IgE antibody have a much lower proportion of cells which also produce γ_3 (P. Mongini, personal communication). This would suggest that $\mu \rightarrow \alpha$ and $\mu \rightarrow \epsilon$ switches within B cell clones are not necessarily preceded by an initial $S_{\mu} \rightarrow S_{\gamma_3}$ event. This latter finding nicely agrees with the extensive S_{μ} sequence homology shared by both S_{ϵ} and S_{α} which would presumably obviate the need for S_{γ_3} participation in successive switching to the C_{ϵ} and C_{α} genes. However, successive, but not necessarily stepwise, isotope switching has been observed in B cell lines which eventually express IgA (39).

We consider the idea of S_{γ_3} participation in successive C_H switching a working hypothesis. Results obtained with spontaneously arising class switch variants clearly indicate that B cells have the

potential to undergo successive C_H gene switches (11-24). We also believe that the excellent correlation between the results presented here and the cellular data on the TNP ficoll immune response (25) is a compelling argument for such a model. Indeed, one could also argue that C_H switching in other T cell independent immune responses may occur through $S_{\gamma 3}$ participation. An excellent way to test part of our hypothesis would be to search for footprints or vestiges of $S_{\gamma 3}$ in the switch-recombination sites of functionally rearranged $\gamma 1$, $\gamma 2b$ or $\gamma 2a$ genes obtained from hybridomas prepared with B lymphocytes responding to TNP-ficoll or a T cell independent mitogen like lipopolysaccharide (LPS).

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