2304–2309 *Nucleic Acids Research, 1999, Vol. 27, No. 11*

The role of components of recombination signal sequences in immunoglobulin gene segment usage: a V81x model

Mani Larijani*, Calvin C. K. Yu+, Rachel Golub, Queenie L. K. Lam and Gillian E. Wu

Department of Immunology and Ontario Cancer Institute, University of Toronto, Room 8-111, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada

Received February 17, 1999; Revised and Accepted April 16, 1999

DDBJ/EMBL/GenBank accession nos AF035204–AF035206

ABSTRACT

It has long been appreciated that some immunoglobulin (and T-cell receptor) gene segments are used much more frequently than others. The V_H segment V81x is a particularly striking case of overusage. Its usage varies with the stage of B-cell development and with the strain of mice, but it is always high in B cell progenitors. We have found that the coding sequence and the recombination signal sequences (RSS) are identical in five mouse strains, including CAST/Ei, a strain derived from the species *Mus castaneus.* Thus, the strain differences cannot be attributed to sequences within V81x itself. V81x RSS mediated recombination at rates significantly higher than another V_H RSS. Although the V81x nonamer differs at one base pair from the consensus sequence, an RSS with this nonamer and a consensus heptamer recombines as well as the consensus RSS. When the V81x spacer is replaced by that of VA1, the frequency of recombination decreases by ~5-fold; thus, the contribution of variation in natural spacers to variability in V_H usage *in vivo* is likely to be more than has been previously appreciated. Furthermore, the contribution of the heptamer and nonamer to differential V_H usage in our assay is correlated inversely with their conservation throughout the V_H locus.

INTRODUCTION

Over-expression of D-proximal V_H gene segments has been well documented in neonatal and fetal mice (1–3). The most strikingly overused V_H segment is V81x, which, depending on the mouse strain, is used in up to 50% of primary pre-B cells (4–12). BALB/c pre-B cells use V81x about twice as frequently as those from C57BL/6 (2,4,13). Its usage falls in mature B cells of all strains with only ~5% of adult spleen B cells using this V_H gene segment (4,5). It has been suggested that the general inability of germline V81x heavy (H) chains to pair with λ5 surrogate light (L) chains is at least partially responsible for this sharp decline of V81x usage as B cells progress towards maturity (14,15).

Despite the variation with mouse strain and with developmental state, V81x is clearly overused. The basis for this overuse is not only an interesting molecular biological puzzle, it is important for understanding how the primary repertoire—the collection of antibody V regions before antigenic selection—is shaped.

It was long presumed that the overusage of V81x was mainly a result of its proximity to the stretch of DNA bearing the D gene segments (2). This presumption has largely been replaced by a general belief that specific accessibility to recombination rather than proximity *per se* is more important for overusage (16–18). It has also been argued that the unusually large number of encoded basic amino acid residues may enable a V81x heavy chain to interact with special antigens, thereby giving this gene segment a unique and pivotal role in the immune system (19). That is, selection of the gene product would be partially responsible for its overusage. However, a V81x knockout strain has no deleterious lymphoid phenotype (18), and this result brings into question the importance of the V81x-encoded protein in the immune system.

Here we present studies directed at clarifying the roles of particular components of the V81x gene segment in its overusage. We demonstrate that the strain differences in V81x usage cannot be attributed to sequences within the gene segment itself, because the coding and the recombination signal sequences (RSS) are identical in five mouse strains, including CAST/Ei, a strain derived from the species *Mus castaneus*. We find that deletion of the entire V81x coding sequence from a microlocus vector does not affect the degree of its overusage. Thus, the RSS, shown by us and others to be an important determinant of the repertoire of Ig H and L chain V-gene segments *in vivo* (20–25), is the *cis* element that is largely responsible for the overusage of V81x. The RSS consists of a nonamer and a heptamer separated by a spacer, which for H-chain V segments is 23 (or 22) bp long. Aside from its role in enforcing the so-called 12/23 rule, which ensures that V recombines with D and not with J, the role of the spacer in determining recombination frequencies is not widely recognized.

*To whom correspondence should be addressed. Tel: +1 416 946 4501; Fax: +1 416 946 2086; Email: manil@oci.utoronto.ca

+Present address: Hooper Foundation, HSW Room 1517, University of California, 513 Parnassus, San Francisco, CA 94143-0552, USA

We previously reported a small (<20%) effect of changing 1 bp in a natural spacer (26), and others have reported larger effects with artificial spacer sequences (21,25,27). Here we find that the natural spacer of V81x is a major determinant of its overusage.

MATERIALS AND METHODS

DDBJ/EMBL/GenBank accession numbers for V81x

C57Bl/6, AF035203; C3H, AF035204; 129, AF035205; CAST/Ei, AF035206.

Germline V81x sequences

Mice were obtained from Jackson Laboratories (ME). DNA was purified by standard procedures and germline V81x was amplified with degenerate 5′ primer VHall (AGGTSMARCTGCAGSAGTCWGG), which hybridizes to V_H codons 1–8 and back primer VH81x3′, which begins 55 bp downstream of the V81x RSS (ATTTCACAACTCTGTGTCTCTCCGC). After a hot start at 94°C for 2 min, 30 cycles of a 3-step PCR (94°C for 1 min, 60°C for 1 min, 72°C for 2 min) was followed by 72°C for 10 min. The PCR product of ~390 bp was cloned with the TA cloning kit (Invitrogen) and for each strain, two to five independent plasmids were sequenced with the T7 sequencing kit (Pharmacia). DNA was amplified and cloned on different days for each mouse strain.

Construction and assay of plasmid substrates

Plasmids were generated from pV81x-D-J microloci (16). The 5′ sense primers were:

V81xRSS GGATCCCACAATGAGCAAAAGTTACTGTGAG
VA1RSS GGATCCCACAGTGTTGTAACCACATGGTGAG
ML11 GGATCCCACAGTGAGCAAAAGTTACTGTGAGCTCAAACAAAAACC
ML12 GGATCCCACAATGAGCAAAAGTTACTGTGAGCTCAAACAAAACC
ML13 GGATCCCACAGTGAGCAAAAGTTACTGTGA
ML14 GGATCCCACAATGTTGTAACCACATCCTGAGTGTGTACTAAACCTCCGATCCTC

The 3′ antisense primer hybridized downstream of the J_H1 segment in the polyoma region: CAACGAAGAGGTCCCTACT. After a hot start at 85°C for 5 min, 30 cycles of a 3-step PCR (94°C for 30 s, 55°C for 1 min and 72°C for 1 min + 5 s per cycle) was followed by 72°C for 10 min. The PCR products of 1.1 kb were cloned into the PCR II vector (Invitrogen TA cloning kit), mapped by restriction digests and sequenced from each end. The verified products were cloned into the backbone of pJC119 (22) via the flanking *Bam*HI sites. The orientation of the inserts in the final constructs were determined by restriction digestion and sequencing.

The microlocus recombination assay *in vitro* has been described (28).

Analysis of DNA populations

Transfection of Abelson murine leukemia virus (A-MuLV) transformed pre-B cell line 1–8 was carried out as described previously (16). Briefly, 1 µg DNA was used to transfect 2×10^7 1–8 cells by the DEAE–Dextran method. DNA recovered from transfections was treated with *Dpn*I to digest non-replicated plasmid DNA, then transfected into ElectroMax DH10B competent bacteria (Gibco-BRL) by electroporation with a GenePulser (Bio-Rad). Typically, we treated half the recovered DNA with *Dpn*I, of which 1–10% was transformed into 25 µl competent bacteria. Transformants were amplified for 16 h in an additional 4 ml LB containing 100 µg/ml ampicillin. Plasmids were recovered by alkaline lysis and digested sequentially with *Nco*I and *Hin*dIII to release the insert from the vector. The resultant DNA fragments were fractionated by gel electrophoresis and analyzed on Southern blots with oligomer probes according to the manufacturer's suggestions (Hybond N, Amersham). The microlocus and its various rearrangement products differed sufficiently in size to identify all recombinants generated by deletion with probe 3′J1CY complementary to J_H1 (16). Bands were quantified on a PhosphorImager (Molecular Dynamics) with ImageQuant 3.3, a method we have quantitatively validated (16). In all experiments, the compared constructs contain the insert in the same orientation (arbitrarily designed 'plus' and 'minus') with respect to the substrate backbone.

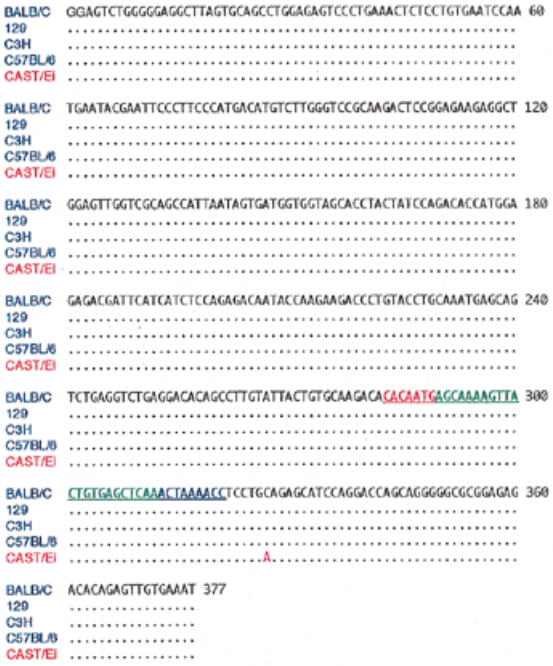

Figure 1. Nucleotide sequence of the V81x gene segment including the nine most 3′ nt of the VHall degenerate primer and the full V81x 3′ primer. Dots indicate identity with the previously published BALB/c sequence (2). The RSS is underlined. The heptamer is shown in red, the spacer in green and the nonamer in blue. The single nt difference in CAST/Ei is shown in red.

RESULTS AND DISCUSSION

V81x coding and RSS sequences are conserved in five strains of mice

Figure 1 shows the germline V81x sequences amplified and cloned from five laboratory strains of mice: BALB/c, 129, C3H, C57BL/6 and CAST/Ei. Like most laboratory mice, the first four strains were derived from *Mus musculus*; CAST/Ei was derived from another species, *M.castaneus*. The sequence conservation is striking, the only difference being at nt 327, 6 nt 3′ of the RSS, where there is an A residue in the CAST/Ei sequence and a C in the other four strains. All other nucleotides are identical. We find in all strains a C at position 336 (15 bp downstream of the RSS) in place of the previously reported T (2). Confirming the report

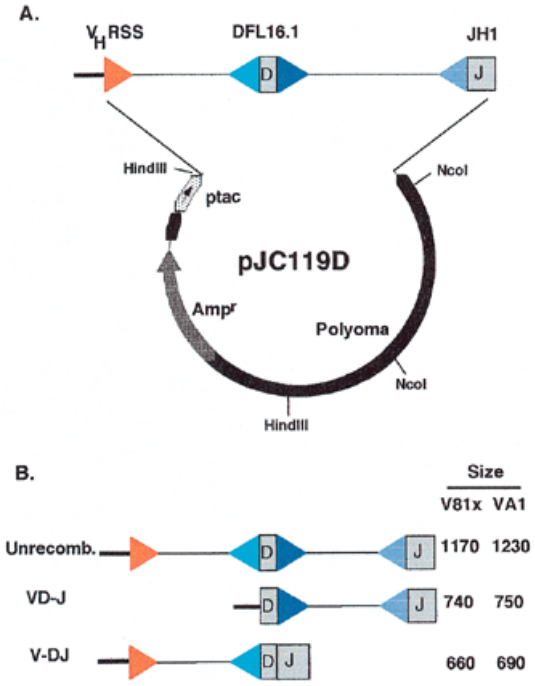

Figure 2. (**A**) Diagram of the vector with the microlocus. (**B**) Relevant deletional products of V(D)J recombination at the microlocus. The microlocus is released from the vector by digestion with *Hin*dIII and *Nco*I.

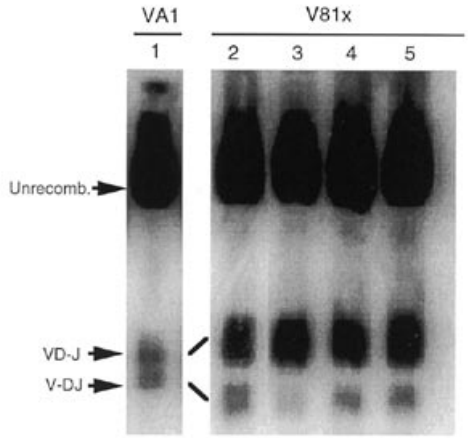

Figure 3. Southern blot analysis of recombinant VA1 and V81x microloci. Products of $V_{RSS}D$ and DJ recombination as well as unrecombined microloci are indicated. All microloci contain the DFL16.1 and J_H1 coding sequences and RSS but vary at the V_HRSS as detailed in Table 2. The analysis is shown in Table 1. Lane 1, pAAA; lane 2, p888; lane 3, pC88; lane 4, p88C; lane 5, pC8C. Products of VD and DJ recombination as well as unrecombined microloci are indicated.

of Holmberg's group (6), we find that codon 15 encodes a glycine (GGA), rather than an arginine (AGA) residue, as was originally reported. We also confirm the conservation of the V81x coding sequences in C57BL/6 and NOD mice (7). Novel and relevant to the present context is the finding that the RSS are identical in all strains studied. That is, strain differences in V81x usage cannot be explained by RSS differences. Thus, the variable usage of a given gene segment among populations, a characteristic central for adaptive immunity, can be due to RSS-independent as well as RSS-dependent mechanisms. As an example of the latter, Feeney and her group have found that usage of the same Vκ gene in different human populations is correlated with variable RSS sequences (29).

The V81x coding sequence does not contribute to its overusage

We showed previously that the VA1 segment (a V_HJ558 family member) recombined less frequently than V81x in microlocus plasmids containing V, D and J segments with full coding sequences and RSS (16). For all the experiments reported here, we constructed a set of 'RSS microloci', all containing the full DFL16.1 and J_H1 segments, but only V_H RSS in place of the full V_H segment (Fig. 2A). That is, the coding region of the V_H gene segments have been removed and vector sequences now abut the RSS. These new coding flanks are identical in both the V81x and VA1 RSS plasmids and are the *Bam*HI restriction site, GGATCC. Figure 2B shows the most abundant deletion products of V(D)J recombination of these substrates, analyzed in this study. Figure 3 shows a typical Southern blot analysis of recombinants. Each lane contains the products, either unrecombined or recombined, of a transfection of one microlocus substrate (see analysis of DNA populations, Materials and Methods). The V_H RSS are the only variable sequence among the microloci. As the D and J sequences are identical in all microloci, the frequency of the DJ recombinants is equal in both plasmids and comparable with those found in our previous studies in which a full V_H sequence was present (16). This constancy allows the frequency of the DJ recombinants to serve as an internal normalization factor for the degree of recombination. The ratio of VD to DJ recombinants which we refer to as the recombination quotient or Q value, thus, can be used as a measure of the relative recombinability of each V_H RSS.

The results from five independent transfection experiments in which the V81x and VA1 RSSs were compared are shown in lines 1 and 5 of Table 1. Lanes 1 and 2 of Figure 3 show recombinants from a representative transfection. On average, the V81x RSS was >6-fold more efficient than the VA1 RSS, a value comparable with results in previous work with V_H segments containing entire coding sequences (16). Coding sequences immediately upstream of the heptamer of the RSS (the coding flanks) have been shown to affect frequency of recombination (25,30–34); however, as the overusage in this assay was mediated by the RSS and many germline V_H segments have the V81x coding flank, CA, we conclude that the coding sequence of V81x cannot be an important contributor to initial V81x overuse.

Conservation of J_H proximal V_H segments among primates has been reported by Meek and colleagues (35). Schroeder and colleagues (36) have also reported conservation within RSS elements, comparing mouse V_HJ558 and V_H7183 with human V_H1 and V_H3 families, respectively. Moreover, neither the V81x knockout mice nor the V81x transgenic mice have adverse phenotypes (18,19), so the protein sequence encoded by V81x does not seem to be essential for B cell development. Our result described in the previous paragraph signifies that the V81x coding sequence does not play a role in the overuse of this segment.

Table 1. Recombination quotients[a] (Q values) of the nonamer and heptamer variants

Source of RSS element				Experiment					
7mer	Spacer	9mer	Name	#1	#2	#3	#4	#5	Average
V81x	V81x	V81x	p888	10	4.1	5.3	9.2	5.6	6.8
V81x	V81x	cons.	p88C	–	–	14	11	15	13
cons.	V81x	V81x	pC88	–	–	25	23	16	21
cons.	V81x	cons.	pC8C	–	13	17	13	26	17
VA1	VA1	VA1	pAAA	1.0	1.0	1.0	1.2	1.0	1.0

[a]Bands corresponding to V(D)J recombination products on Southern blot gels were quantified with a PhosphorImager. The recombination quotient (Q value) is defined to be the ratio of VD to DJ products. As D and J are the same in all experiments, the Q value is a measure of the recombinability of a V element. The actual sequences for V81x, VA1 and consensus heptamers and nonamers, as well as V81x and VA1 spacers are given in Table 2. The relative orientation of all variant vectors are 'minus'. cons., consensus; –, not done.

Functional analysis of the V81x RSS heptamer and nonamer

Of all known RSS sequences, the consensus is the most competent in reporter assays (27,37). As shown in Figure 1 and Table 2, the heptamer and nonamer of the V81x RSS each deviate from the consensus sequence by one nucleotide. In order to examine recombinational competence of the V81x RSS relative to the consensus RSS, we systematically made plasmids with all four combinations of V81x and consensus heptamers and nonamers, while maintaining the V81x 23 bp spacer. The sequence of the variant RSS in each microlocus is shown in Table 2. A summary of results from several independent transfection experiments with these plasmids is shown in Table 1. Figure 3 shows a representative Southern blot used in quantification. Changing either the heptamer or the nonamer of V81x to consensus improved the strength of the V81x RSS 2–3-fold (Table 1, lines 2 and 3). However, changing both residues back to consensus did not show an additive effect (Table 1, line 4). A microlocus with a consensus heptamer and a V81x nonamer yielded as many or more recombinants than one with consensus heptamer and nonamer (Table 1, lines 3 and 4,).

Table 2. RSS sequences in the microlocus plasmids

Source of RSS element			Sequence			
7-mer	Spacer	9-mer	Heptamer	Spacer	Nonamer	Name
V81x	V81x	V81x	CACAATG	AGCAAAAGTTACTGTGAGCTCAA	ACTAAAACC	p888
V81x	V81x	cons.	CACAATG	AGCAAAAGTTACTGTGAGCTCAA	ACAAAAACC	p88C
cons.	V81x	V81x	CACAGTG	AGCAAAAGTTACTGTGAGCTCAA	ACTAAAACC	pC88
cons.	V81x	cons.	CACAGTG	AGCAAAAGTTACTGTGAGCTCAA	ACAAAAACC	pC8C
VA1	VA1	VA1	CACAGTG	TTGTAACCACATCCTGAGTGTGT	CAGAAACCC	pAAA
V81x	VA1	V81x	CACAATG	TTGTAACCACATCCTGAGTGTGT	ACTAAAACC	p8A8

Modifications from the V81x RSS are shown in red.

Previous analyses have examined in some detail the effect of non-consensus nucleotides on V(D)J recombination (24,27,29). When tested with either a 12 or 23 bp spacer, the recombination frequency decreased 15–27%, if the fifth nucleotide of the heptamer contained a non-consensus A, as in V81x (37). The V81x nonamer, in which the third nucleotide is a T instead of an A, has not been tested in any recombination assay. However, when Akamatsu *et al*. tested a G at that position in a 12 bp spacer RSS with a consensus heptamer, recombination was decreased by 57% (27). Thus the previous analyses did not foretell the recombination strength of the V81x RSS elements.

Functional analysis of the V81x RSS spacer

The puzzlingly high frequency of recombination (up to 20%) in the V81x RSS variant microloci led us to examine the only common element among these sequences: the V81x spacer. A microlocus containing the V81x heptamer and nonamer, but with the VA1 spacer, was constructed and assayed. A representative Southern blot is shown in Figure 4. As shown in Table 3, the RSS containing the VA1 spacer mediated recombination several-fold less frequently than the RSS containing the V81x spacer sequence. When spacers of actual gene segments are compared, this is, to our knowledge, the largest spacer effect on V(D)J recombination reported to date (21,25,26,38).

Table 3. Q values of the spacer variants

Source of RSS element				Experiment			
7mer	Spacer	9-mer	Name	#6	#7	#8	Average
V81x	V81x	V81x	p888	21	8.2	9.1	13
V81x	VA1	V81x	p8A8	4.3	3.0	2.2	3.2

The description is the same as in Table 1. The relative orientation of both variant vectors are 'plus'.

The spacer was once thought to be just that, a determinant of distance between the heptamer and nonamer, thereby maintaining the 12/23 rule. Nevertheless, spacer lengths can vary by one nucleotide (12 ± 1 or 23 ± 1 bp) and these variations significantly affect recombination frequencies (27,37). The spacer sequence shows limited conservation at some positions (39), but substitution studies aimed at determining the functional significance of this conservation found no specific effects in some cases (25,27,40), while in other cases specific effects were seen (21,26,38). Reports of studies where the spacer lengths were increased by multiples of the basic ~11 bp unit also concluded that the 12/23 pair were the most efficient.

Our finding that replacement of the V81x spacer with the VA1 spacer—two naturally occurring spacers—reduces recombination

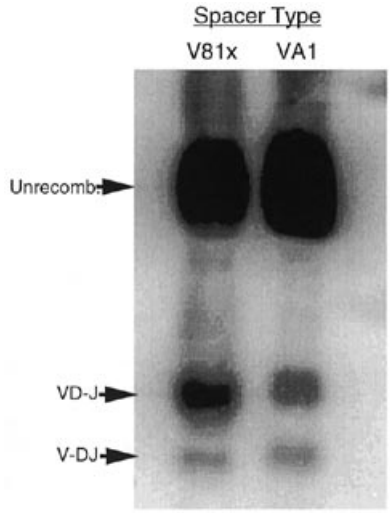

Figure 4. Southern blot analysis of recombinant microloci differing only in the spacer. Both microloci contain the same D and J as in Figure 3. Both contain the RSS nonamer and heptamer from V81x. The spacer is from the V segment indicated. Lane V81x is p888; lane VA1 is p8A8. The sequences are shown in Table 2, the analysis in Table 3. Products of VD and DJ recombination as well as unrecombined microloci are indicated.

frequencies several-fold shows that the V81x spacer is an intriguingly strong component of the overall strength of the V81x RSS. Unique to the V81x spacer is a track of four As in its heptamer proximal half where one group reports of RAG protein contacts (41) . We also note that though comparable in their GC content, the V81x and VA1 spacers differ at their nonamer proximal residues, the position another group reports RAG1 may contact (42). Our data extend such biochemical observations to the level of functional differences in gene usage *in vivo*. We suggest that 23 bp spacers contribute to differences in V_HRSS function as well as maintaining the order of rearrangement in the IgH locus through the 12/23 rule.

Comparison of heptamer and nonamer variability

Biochemical studies suggest that heptamer and nonamer may play distinct roles in V(D)J recombination. All reports suggest that the heptamer marks the precise position of the V(D)J cleavage sites by a RAG1/2 complex (41,43–48). While some reports suggest that the nonamer is a RAG1 recruiting and initial binding element (43,44,46), others indicate that when both RAG1 and RAG2 are present, the nonamer, spacer and spacer proximal nucleotides of the heptamer are all bound (41,47). We await elucidation as to what mechanism is in effect *in vivo*. Nevertheless, the lack of additivity of the effects of heptamer and nonamer is eminently compatible with the notion of their having distinct roles.

In this regard, it is interesting that there is more variation in the nonamer among V_H segments than in the heptamer sequences. Previously, there had been no reported examples of a V_H segment in mice with both consensus heptamer and nonamer sequences. We have isolated a V_H gene segment with such a consensus V_H RSS. This V_H segment belongs to the V_HQ52 family which is interspersed with the V_H7183 family of V_H genes.

Among a panel of 33 V_H gene segments (Table 4) (39), the heptamers were consensus in all but three, including the V81x RSS (average of 0.09 non-consensus nt in each heptamer). Presumably the heptamer conservation is necessary to ensure precise cleavage sites for the recombination machinery (46). A precise heptamer would favor a given sequence at the V_H border, which might be especially important in early fetal development when there is no N addition (49). On average, V_H RSS nonamers differ from the consensus by 1.8 nt. Indeed the most common nt in position 4 for both mouse and human V_H nonamers is G or C, not A. Thus, V_H RSS nonamers are more variable than heptamers. In D_H and J_H, on the other hand, nonamer and heptamer variability is similar: 0.55 and 0.70 non-consensus nt per heptamer and nonamer element for D_H and 1.3 and 1.3 nt for J_H (Table 4). All D_H spacers are 12 bp long. However, two of the four J_H spacers and only 11 out of the 33 V_H segments had a 23 bp spacer; the others had a less effective 22 bp spacer (27,37).

Table 4. Variation[a] of Ig H-chain RSS heptamers and nonamers

	V_H	D	J_H
Heptamer	0.090	0.55	1.3
Nonamer	1.8	0.70	1.3

[a]The average number of differences in the RSS heptamer or nonamer of 33 V_H and all known D and J_H segments from the consensus heptamer or nonamer RSS (39). The values are the average number of nucleotide changes per element.

Compared with other V_H segment RSS, the V81x RSS is unusual. All components of the V81x RSS influence the frequency at which it is used in recombination reporter constructs and thus probably contribute to the overusage of V81x *in vivo*. It is possible that the conserved V81x RSS has evolved to mediate a given initial rate of V81x to DJ_H joining most advantageous for B cell development. For instance, there may be advantages conferred by V81x to D deletional recombination, such as serving to activate or 'open' the locus. Like the spacer, the nonamer can and does vary, and together these two elements generate most of the variability in RSS strength in our microlocus plasmids. Based on the RSS sequence analysis, we suggest that the nonamer and spacer are likely to be the primary determinants of recombination efficiency of V_H segments *in vivo*.

ACKNOWLEDGEMENTS

We thank Drs Charley Steinberg, Chris Paige and Susanna Lewis for helpful discussions and help with the manuscript. This work was supported by grants from the National Cancer Institute of Canada, the Terry Fox Marathon of Hope and the Medical Research Council of Canada. M.L. holds an MRC studentship award and G.E.W. is an MRC Scientist.

REFERENCES

1 Perlmutter,R.M., Kearney,J.F., Chang,S.P. and Hood,L.E. (1985) *Science*, **227**, 1596–1601.
2 Yancopoulos,G.D., Desiderio,S.V., Paskind,M., Kearney,J.F., Baltimore,D. and Alt,F.W. (1984) *Nature*, **311**, 727–733.
3 Malynn,B.A., Yancopoulos,G.D., Barth,J.E., Bona,C.A. and Alt,F.W. (1990) *J. Exp. Med.*, **171**, 843–859.
4 Marshall,A.J., Wu,G.E. and Paige,C.J. (1996) *J. Immunol.*, **156**, 2077–2084.

5 Marshall,A.J., Paige,C.J. and Wu,G.E. (1997) *J. Immunol.*, **158**, 4282–4291.
6 Carlsson,L., Övermo,C. and Holmberg,D. (1992) *Eur. J. Immunol.*, **22**, 71–78.
7 Andersson,A., Ekstrand-Hammarstrom,B., Eriksson,B., Overmo,C. and Holmberg,D. (1994) *Int. Immunol.*, **6**, 623–630.
8 Hardy,R. (1992) *Curr. Opin. Immunol.*, **4**, 181–185.
9 Freitas,A., Andrade,L., Lembezat,M.-P. and Coutinho,A. (1990) *Int. Immunol.*, **2**, 15–23.
10 Huetz,F., Carlsson,L., Tornberg,U. and Holmberg,D. (1993) *EMBO J.*, **12**, 1819–1826.
11 Reth,M., Jackson,S. and Alt,F.W. (1986) *EMBO J.*, **5**, 2131–2138.
12 Viale,A.-C., Coutinho,A. and Freitas,A.A. (1992) *J. Exp. Med.*, **175**, 1449–1465.
13 Kearney,J., Bartels,J., Hamilton,A., Lehuen,A., Solvason,N. and Vakil,M. (1992) *Int. Rev. Immunol.*, **8**, 247–257.
14 ten Boekel,E., Melcher,F. and Rolink,A. (1997) *Immunity*, **7**, 357–368.
15 Keyna,U., Beck-Engeser,G., Jongstra,J., Applequist,S. and Jack,H.-M. (1995) *J. Immunol.*, **155**, 5536–5542.
16 Yu,C.C., Larijani,M., Miljanic,I.N. and Wu,G.E. (1998) *J. Immunol.*, **161**, 3444–3454.
17 Alt,F., Yancopolous,G., Blackwell,T.K., Wood,C., Thomas,E., Boss,M., Coffman,R., Rosenberg,N., Tonegawa,S. and Baltimore,D. (1984) *EMBO J.*, **3**, 1209–1219.
18 Huetz,F., Tornberg,U., Malanchère,E., Roes,J., Carlsson,L., Coutinho,A., Holmberg,D. and Rajewsky,K. (1997) *Eur. J. Immunol.*, **27**, 307–314.
19 Martin,F., Chen,X. and Kearney,J.F. (1997) *Int. Immunol.*, **9**, 493–505.
20 Ramsden,D.A. and Wu,G.E. (1991) *Proc. Natl Acad. Sci. USA,.*, **88**, 10721–10725.
21 Nadel,B., Tang,A., Escuro,G., Lugo,G. and Feeney,A.J. (1998) *J. Exp. Med.*, **187**, 1495–1503.
22 Connor,A., Fanning,L., Celler,J., Hicks,L., Ramsden,D. and Wu,G. (1995) *J. Immunol.*, **155**, 5268–5272.
23 VanDyk,L.F., Wise,T.W., Moore,B.B. and Meek,K. (1996) *J. Immunol.*, **157**, 4005–4015.
24 Pan,P., Lieber,M. and Teale,J. (1997) *Int. Immunol.*, **9**, 515–522.
25 Wei,Z. and Lieber,M. (1993) *J. Biol. Chem.*, **268**, 3180–3183.
26 Fanning,L., Connor,A., Baetz,K., Ramsden,D. and Wu,G. (1996) *Immunogenetics*, **44**, 146–150.
27 Akamatsu,Y., Tsurushita,N., Nagawa,F., Matsuoka,M., Okazaki,K., Imai,M. and Sakano,H. (1994) *J. Immunol.*, **153**, 4520–4529.
28 Connor,A., Fanning,L.J., Bentolila,L.A. and Wu,G.E. (1997) *Immunol. Methods Manual*, **4**, 259–268.
29 Nadel,B., Tang,A., Lugo,G., Love,V., Escuro,G. and Feeney,A.J. (1998) *J. Immunol.*, **161**, 6068–6073.
30 Sadofsky,M., Hesse,J., van Gent,D. and Gellert,M. (1995) *Genes Dev.*, **9**, 2193–2199.
31 Gerstein,R. and Leiber,M. (1993) *Genes Dev.*, **7**, 1459–1469.
32 Ezekiel,U., Engler,P., Stern,D. and Storb,U. (1995) *Immunity*, **2**, 381–389.
33 Chukwuocha,R. and Feeney,A. (1993) *Mol. Immunol.*, **30**, 1473–1480.
34 Nadel,B. and Feeney,A.J. (1995) *J. Immunol.*, **155**, 4322–4329.
35 Meek,K., Eversole,T. and Capra,J. (1991) *J. Immunol.*, **146**, 2434–2438.
36 Schroeder,H.W.,Jr, Hillson,J.L. and Perlmutter,R.M. (1989) *Int. Immunol.*, **2**, 41–50.
37 Hesse,J., Lieber,M., Mizzuuchi,K. and Gellert,M. (1989) *Genes Dev.*, **3**, 1953–1961.
38 Posnett,D.N., Vissinga,C.S., Pambuccian,C., Wei,S., Robinson,M.A., Kostyu,D. and Concannon,P. (1994) *J. Exp. Med.*, **179**, 1707–1711.
39 Ramsden,D.A., Baetz,K. and Wu,G.E. (1994) *Nucleic Acids Res.*, **22**, 1785–1796.
40 Akira,S., Okazaki,K. and Sakano,H. (1987) *Science*, **238**, 1134–1138.
41 Swanson,P.C. and Desiderio,S. (1998) *Immunity*, **9**, 115–125.
42 Nagawa,F., Ishiguro,K., Tsuboi,A., Yoshida,T., Ishikawa,A., Takemori,T., Otsuka,A.J. and Sakano,H. (1998) *Mol. Cell. Biol.*, **18**, 655–663.
43 Cuomo,C.A., Mundy,C.L. and Oettinger,M.A. (1996) *Mol. Cell. Biol.*, **16**, 5683–5690.
44 Difilippantonio,M.J., McMahan,C.J., Eastman,Q.M., Spanopoulou,E. and Schatz,D.G. (1996) *Cell*, **87**, 253–262.
45 Spanopoulou,E., Zaitseva,F., Wang,F.H., Santagata,S., Baltimore,D. and Panayotou,G. (1996) *Cell*, **87**, 263–276.
46 Ramsden,D., McBlane,J., van Gent,D. and Gellert,M. (1996) *EMBO J.*, **15**, 3197–3206.
47 Akamatsu,Y. and Oettinger,M.A. (1998) *Mol. Cell. Biol.*, **18**, 4670–4678.
48 Kim,D.R. and Oettinger,M.A. (1998) *Mol. Cell. Biol.*, **18**, 4679–4688.
49 Feeney,A.J. (1990) *J. Exp. Med.*, **172**, 1377–1390.