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Structural relationships among mouse and human immunoglobulin V_H genes in the subgroup III

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ABSTRACT

The mouse VHIII subgroup is composed of four families which share sequence homology. We isolated a VH germ-line genomic clone, which cross hybridizes with a cDNA probe from one of these families, derived from a myeloma secreting an antigalactan antibody. We report here the nucleotide sequence of the cross hybridizing gene and show that very likely it has an anti-sheep red blood cell specificity. Comparison of its nucleotide sequence with those of the three other VHIII families shows that these genes share segmental homologies of variable lengths. This suggests that interchanges of sequence blocks between VH genes could be an important evolutionary mechanism for diversifying the germ-line repertoire. The strong homology (82 %) with human VHIII genes suggests that efficient antibody sequences are strongly conserved. This conservation of homology is particularly striking when compared to the more limited homology (63 %) between mouse and human Cκ genes.

INTRODUCTION

The extensive repertoire of immunoglobulin variable regions can be accounted for in three ways : (i) the multiplicity of separate germ-line genes for each different segment of heavy chains (V_H, D, J_H) and light chains (V_L, J_L) ; (ii) the combinatorial joining of the different gene segments to produce a complete V region and (iii) somatic diversification.

In the mouse, the VH regions can be divided into five subgroups, each of which is defined by a characteristic amino acid sequence as well as shared sequence insertions or deletions (1). The VHIII subgroup is composed of at least four families, the prototypes of which have respectively antigalactan, anti-inulin, antiphosphorylcholine and anti-sheep red blood cell specificities.

The rapid accumulation of data from the mouse VHIII germ-line sequences provides important insights on the molecular mechanisms that may play a role in the establishment and evolution of this multigene family. We have previously reported the nucleotide sequence of a mouse VHIII germ-line gene (VH441) which codes for antigalactan myeloma proteins (X44, T601) (2).

We have now isolated and sequenced another gene (VH283) which is related to VH441. In this paper, we compare this sequence to other previously published sequences and make the following observations. An antibody most probably encoded by gene VH283 determines anti-sheep red blood cell specificity. This sequence also suggests that it is genealogically related to a family of the human VHIII subgroup, which underwent a significant amplification (3,4). A comparison of nucleotide sequences from a member of each of the four mouse VHIII families shows segmental homologies among these genes similar to those described by Kabat on the basis of amino acid sequences (5). Using either VH441 or VH283 clone as probe, the same hybridization restriction patterns were detected in BALB/c DNA digested with EcoRI enzyme. The implications of these observations are discussed below.

METHODS

Isolation of recombinant clone

The charon 4A, recombinant bacteriophage library containing BALB/c embryo DNA has been previously described (2). This library was screened with a nick-translated ^{32}P labeled subclone, p325 VH441, according to the method of Benton and Davis (6).

DNA sequencing analysis

A 3.8 kb EcoRI fragment containing the VH283 was subcloned into pBR325. A BstN1 DNA fragment of this subclone was labeled at the 3' end by filling in this protruding restriction site with E.coli DNA polymerase I large fragment, and was used to determine the nucleotide sequence according to the procedure of Maxam and Gilbert (7).
By using a PstI fragment bearing the VH283 gene, M13 clones in both orientations were obtained. This PstI fragment was also used to construct M13 deletion clones using exonuclease Bal31. Segments in M13 were sequenced according to the procedures described by Sanger et al. (8) and Messing et al. (9).

Genomic blot hybridization

DNA was isolated from BALB/c mouse embryo as described by Maniatis et al. (10). DNA was digested by EcoRI, fractionated on a 0.8 % agarose gel, transferred to "Gene Screen" membrane filters (New England Nuclear) and hybridized to ^{32}P probe as described by Southern (11). Final wash after hybridization was in 0.4 x SSC, 0.1 % NaDodSO4 at 68°C.

Probes

The EcoRI insert of p325-VH441-IV clone was used as antigalactan

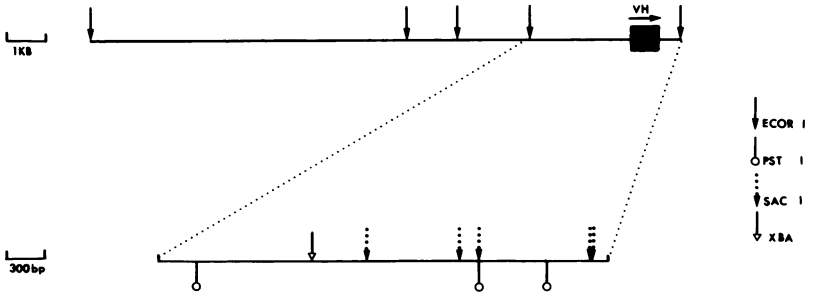


FIGURE 1 : Restriction map of VH283 clone

1) The top line represents the cloned 14.8 kb EcoRI fragments in charon 4A VH283 ;
2) Below is represented a magnified detail of a 3.8 kb EcoRI fragment obtained by the method of Smith and Birnstiel (24).

probe (2). The PstI insert of p325-VH283 clone was used as anti-SRBC probe (this paper).

RESULTS

ISOLATION OF VH CLONE

The λ phage library of BALB/c mouse embryo was screened as previously described with the nick-translated p325-VH441-IV probe (2). Figure 1 shows the restriction map of VH283 clone containing a 14.8 kb insert.

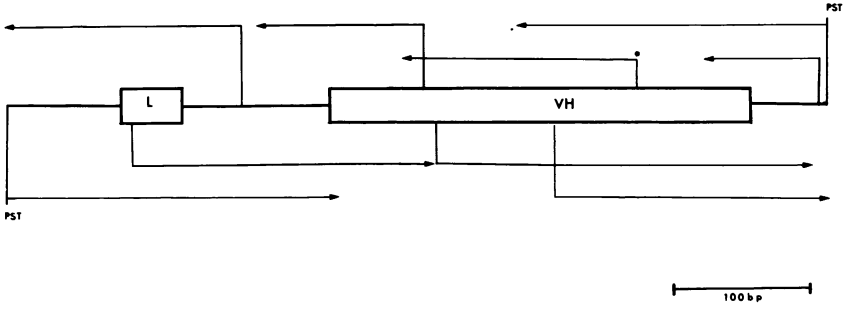
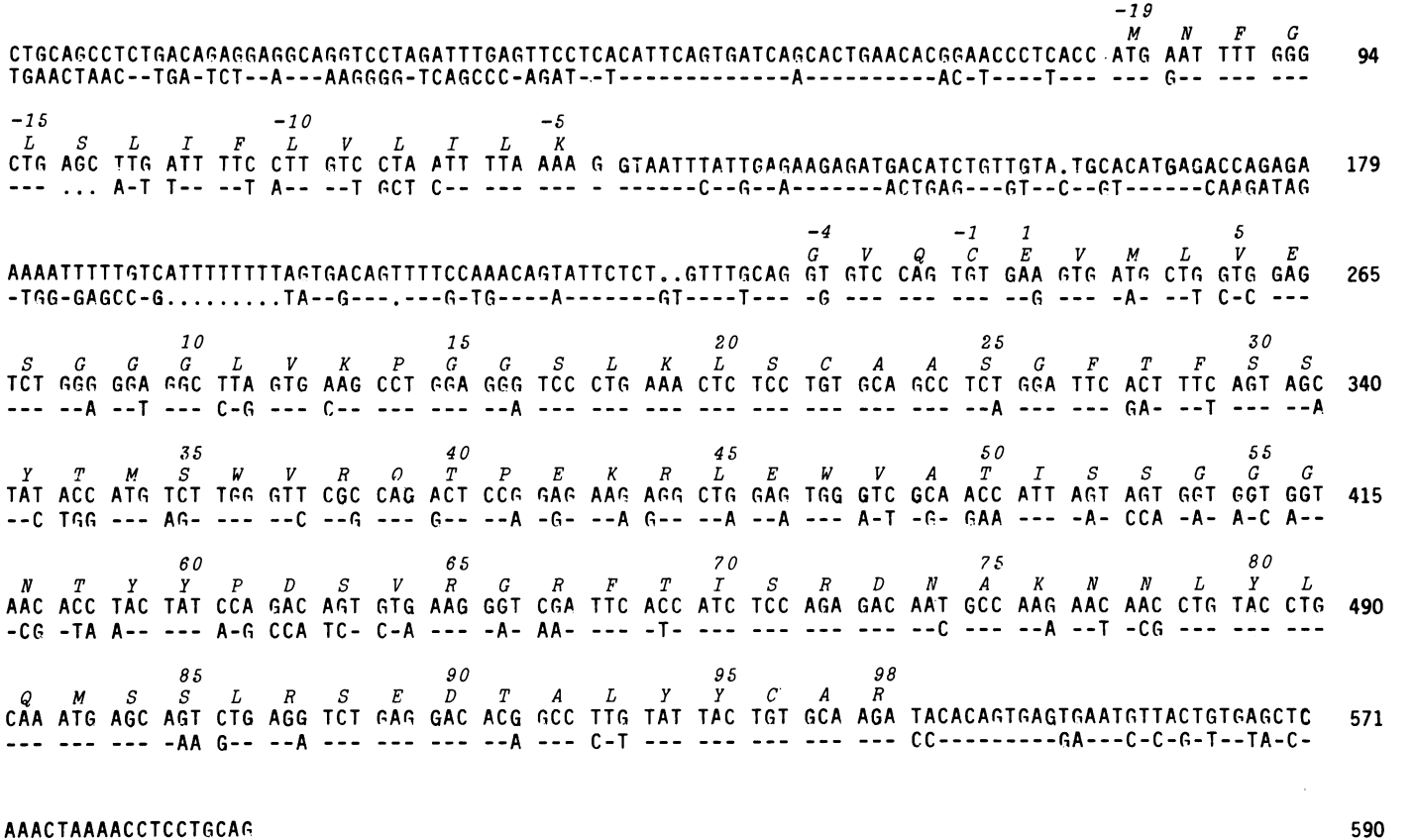


FIGURE 2 : Strategy for sequence determination of VH283 gene

The coding regions of the gene (open boxes), the intervening sequences and the 3' and 5' sequenced flanking regions (solid line) are indicated. The horizontal arrows below and above the map give the 5'→3' direction and the length of individual M13 deletion clones (obtained by Bal31 digestion) sequenced. The horizontal arrow with a star indicates the fragment sequenced by the method of Maxam and Gilbert (7).


-19
M N F G
CTGCAGCCTCTGACAGAGGAGGCAGGTCCTAGATTTGAGTTCCTCACATTCAGTGATCAGCACTGAACACGGAACCCTCACC ATG AAT TTT GGG 94
TGAACTAAC--TGA-TCT--A---AAGGGG-TCAGCCC-AGAT--T----------A----------AC-T----T--- --- G-- --- ---
-15 -10 -5
L S L I F L V L I L K
CTG AGC TTG ATT TTC CTT GTC CTA ATT TTA AAA G GTAATTTATTGAGAAGAGATGACATCTGTTGTA.TGCACATGAGACCAGAGA 179
--- ... A-T T-- --T A-- --T GCT C-- --- --- - ------C--G--A-------ACTGAG---GT--C--GT------CAAGATAG
-4 -1 1 5
G V Q C E V M L V E
AAAATTTTTGTCATTTTTTTTAGTGACAGTTTTCCAAACAGTATTCTCT..GTTTGCAG GT GTC CAG TGT GAA GTG ATG CTG GTG GAG 265
-TGG-GAGCC-G.....TA--G---.---G-TG----A-------GT----T--- -G --- --- --- --G --- -A- --T C-C ---
10 15 20 25 30
S G G G L V K P G G S L K L S C A A S G F T F S S
TCT GGG GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT AGC 340
--- --A --T --- C-G --- C-- --- --- --A --- --- --- --- --- --- --- --- --A --- --- GA- --T --- --A
35 40 45 50 55
Y T M S W V R Q T P E K R L E W V A T I S S G G G T
TAT ACC ATG TCT TGG GTT CGC CAG ACT CCG GAG AAG AGG CTG GAG TGG GTC GCA ACC ATT AGT AGT GGT GGT GGT 415
--C TGG --- AG- --- --C --G --- G-- --A -G- --A G-- --A --A --- A-T -G- GAA --- -A- CCA -A- A-C A--
60 65 70 75 80
N T Y Y P D S V R G R F T I S R D N A K N N L Y L
AAC ACC TAC TAT CCA GAC AGT GTG AAG GGT CGA TTC ACC ATC TCC AGA GAC AAT GCC AAG AAC AAC CTG TAC CTG 490
-CG -TA A-- --- A-G CCA TC- C-A --- -A- AA- --- -T- --- --- --- --- --C --- --A --T -CG --- --- ---
85 90 95 98
Q M S S L R S E D T A L Y Y C A R
CAA ATG AGC AGT CTG AGG TCT GAG GAC ACG GCC TTG TAT TAC TGT GCA AGA TACACAGTGAGTGAATGTTACTGTGAGCTC 571
--- --- --- -AA G-- --A --- --- --- --A --- C-T --- --- --- --- --- CC---------GA---C-C-G-T--TA-C-
AAACTAAAACCTCCTGCAG 590
-G--ATG------ACTGTG

EcoRI digestion of this insert generates four fragments of 1.23 kb, 1.8 kb, 3.8 kb and 8 kb, which have been ordered by digestion with several enzymes alone or in combinations. The UPC10 VH cDNA probe (2) hybridizes to a 600 bp PstI fragment contained in the 3.8 kb EcoRI fragment. This fragment was cloned in the PstI site of M13 mp 7 phage and also used to construct deletion clones by digestion with Bal31 exonuclease as described in Methods. Both types of subclones were used to perform the sequence determination by the dideoxy method of Sanger.

SEQUENCE OF VH283

Figures 2 and 3 show respectively the sequencing strategy and the nucleotide sequence of the 597 bp VH283 PstI fragment compared to VH441. The two genes have an overall 75% and 62,5% homology in the coding and non coding regions respectively. The limited homology in the coding region, which is discussed below, exists as blocks embedded in highly divergent sequences throughout this region. The VH283 leader sequence has the same additional codon AGC (Ser) located between amino acid codon -14 and -13 as was found in the precursor of the phosphorylcholine VH genes (12). In non coding regions, a homologous sequence with six substitutions out of 39 nucleotides at the 5' end of the VH region was located 3' to a very divergent sequence. This homologous sequence preceding the ATG initiation site was identified as the 5' untranslated region because it is included in the cDNA of the VH clone UPC10. Several deletions and insertions affect the length of the intron. The VH283 intron is 108 bp long versus 102 bp for VH441.

VH283 BELONGS TO THE ANTI-SHEEP RED BLOOD CELL FAMILY

We have compared the nucleotide sequence of our genomic clone VH283 with the 5' end of the VH segment of the cDNA clone pμ/107, which was derived from the μ chain RNA of the hybridoma Sp1/HL secreting an anti-sheep red blood cell antibody. This comparison reveals that 113 out of 114 nucleotides (at amino acid position 1-38) are identical to the sequence previously determined (13). The single base substitution is located in the hypervariable one region (HV1) at amino acid position 33 where the codon ACC (Thr) in VH283 replaces GCC (Ala) in the pμ/107 cDNA sequence. Since the single

FIGURE 3 : The DNA sequence of VH283 germ-line gene

Amino acid sequences encoded by the exons of the gene are shown above the DNA sequences of VH283. Homologous nucleotides are indicated by dashes, and deletions required to maximize the homology are indicated by dots.

```
                                                                      1                                            10
                                                              VH283   GAA GTG ATG CTG GTG GAG TCT GGG GGA GGC TTA GTG
                                                              VH441 · GAG GTG AAG CTT CTC GAG TCT GGA GGT GGC CTG GTG
                                                              V1      GAG GTG AAG CTG GTG GAA TCT GGA GGA GGC TTG GTA
                                                              HPC-76                      GAG TCT GGA GGA GGC TTG GTG
                                                              H11     GAG GTG CAG CTG GTG GAG TCC GGG GGA GGC TTA GTT

                                            20                                     30          HV 1
VH283  AAG CCT GGA GGG TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT AGC TAT ACC ATG TCT TGG GTT CGC CAG
VH441  CAG CCT GGA GGA TCC CTG AAA CTC TCC TGT GCA GCC TCA GGA TTC GAT TTT AGT AGA TAC TGG ATG AGT TGG GTC CGG CAG
V1     CAG CCT GGG GGT TCT CTG AGA CTC TCC TGT GCA ACT TCT GGG TTC ACC TTC AGT GAT TTC TAC ATG GAG TGG GTC CGC CAG
HPC-76 CAA CCT GGA GGA TCC ATG AAA CTC TCC TGT GTT GCC TCT GGA TTC ACT TTC AGT AAC TAC TGG ATG AAC TGG GTC CGC CAG
H11    CAG CCT GGG GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAC TGG ATG CAC TGG GTC CGC CAA

               40                                 50                      HV 2    60
VH283  ACT CCG GAG AAG AGG CTG GAG TGG GTC GCA ACC ATT AGT AGT GGT GGT GGT AAC ACC TAC TAT CCA GAC AGT ... ... GTG
VH441  GCT CCA GGG AAA GGG CTA GAA TGG ATT GGA GAA ATT AAT CCA GAT AGC AGT ACG ATA AAC TAT ACG CCA TCT ... ... CTA
V1     CCT CCA GGG AAG AGA CTG GAG TGG ATT GCT GCA AGT AGA AAC AAA GCT AAT GAT TAT ACA ACA GAG TAC AGT GCA TCT GTG
HPC-76 TCT CCA GAG AAG GGG CTT GAG TGG GTT GCT GAA ATT AGA TTG AAA TCT ... GGT TAT GCA ACA CAT TAT GCG GAG TCT GTG
H11    GCT CCA GGG AAG GGG CTG GTG TGG GTC TCA CGT ATT AAT AGT GAT GGG AGT AGC ACA ACG TAC GCG GAC TCC ... ... GTG

                           70                            80                                      90
VH283  AAG GGT CGA TTC ACC ATC TCC AGA GAC AAT GCC AAG AAC AAC CTG TAC CTG CAA ATG AGC AGT CTG AGG TCT GAG GAC ACG
VH441  AAG GAT AAA TTC ATC ATC TCC AGA GAC AAC GCC AAA AAT ACG CTG TAC CTG CAA ATG AGC AAA GTG AGA TCT GAG GAC ACA
V1     AAG GGT CGG TTC ATC GTC TCC AGA GAC ACT TCC CAA AGC ATC CTC TAC CTT CAG ATG AAT GCC CTG AGA GCT GAG GAC ACT
HPC-76 AAA GGG AGG TTC ACC ATC TCA AGA GAT GAT TCC AAA AGT AGT GTC TAC CTG CAA ATG AAC AAC TTA AGA GCT GAA GAC ACT
H11    AAG GGC CGA TTC ACC ATC TCC AGA GAC AAC GCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGT CTG AGA GCC GAG GAC ACG

                                 98
VH283  GCC TTG TAT TAC TGT GCA AGA
VH441  GCC CTT TAT TAC TGT GCA AGA
V1     GCC ATT TAT TAC TGT GCA AGA GAT
HPC-76 GCC ATT TAT TAC TGT ACC AGG CCG
H11    GCT GTG TAT TAC TGT GCA AGA
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base difference is probably due to a somatic mutation, we can confidently assign the germ-line gene VH283 to the anti-SRBC family.

NUCLEOTIDE SEQUENCE COMPARISON OF VHIII GENES FROM FOUR DIFFERENT MOUSE AND ONE HUMAN FAMILIES

Four mouse VHIII prototype sequences were compared over 295-306 nucleotides of coding sequences. See Figure 4. Table 1 shows the homology shared among members of the different families. Although the differences observed are spead throughout the sequence, homologies occur in blocks of 8 to 22 conserved nucleotides. In each species the VHIII subgroup appears to be constituted of families in which members share more than 85% homology at the nucleotide level (12,14,15).

Three human VHIII genes have been previously sequenced (3,4). These genes show 81 to 82,5% homology in the coding sequence with our mouse VH283. Only H11, the most homologous one, was used for comparison in Figure 4 and Table 1. Previous analyses based on comparison of fragmentary data available from amino acid sequences of mammalian VHIII proteins have shown high conservation through the evolution (16,17).

BLOT HYBRIDIZATION ANALYSES OF VH GENE FRAGMENTS RELATED TO ANTI-GALACTAN FAMILY

It was of interest to determine if the size of the VHIII germ-line repertoire in BALB/c mice could be estimated by filter hybridization experiments, using the two genomic clones derived from two different families of this subgroup. BALB/c liver DNA was digested by EcoRI and hybridized either to the VH441 p325-IV subclone belonging to the antigalactan family or to the VH283 Pst fragment from the anti-sheep red blood cell family as probes. Both probes, which are 75,5% homologous by nucleotide sequence comparison, detect the same approximatively ten EcoRI fragments shown in Figure 5. An additional 500 bp fragment, which is contained in the original VH441 clone, is detected after longer exposures but only with the VH441

FIGURE 4 : Nucleotide sequence comparison of VHIII genes from four different mouse and one human families

The five coding sequences are aligned, the dots represent deletions introduced to maximize the homology. Sequence data for human VH gene H11 are from Rechavi et al. (4), for mouse HPC-76 anti-inulin specificity from Bernard and Gough (25), Kemp et al. (26) ; for mouse V1 antiphosphorylcholine specificity from Crews et al. (12) ; for mouse VH441 antigalactan specificity from Ollo et al. (2) and for VH283 anti-SRBC specificity from this paper.

TABLE 1

A - Comparison of nucleotide sequence of VHIII genes from four different mouse families.
B - Comparison of nucleotide sequence between mouse VH283 and human H11 VH genes.

Gene segments	Number of nucleotides compared	% of homology	Silent substitutions	Remplacement
VH283/VH441	345	75.5	37	46
VH283/V1	306	72.9	38	45
VH283/HPC-76	279	71.5	40	40
VH441/HPC-76	279	71.5	37	43
V1/HPC-76	279	75.1	34	37
V441/V1	306	68.5	42	55
VH283/H11	351	82.5	28	33

Deletions introduced to maximize the homology were not taken into account (for a difference in calculation).

probe (data not shown). A corresponding fragment for the VH283 probe would be difficult to detect due to its small size and more limited homology.

DISCUSSION

The data presented is this paper make it possible to address the question of whether a probe for one member of a subgroup family can detect genes from other families in the same subgroup. The VH441 and VH283 clones discussed in this paper were shown to belong to different families of the VHIII subgroup. These genes share an overall homology of only 75% for coding regions and 62,5% for non coding regions. Probes derived from either of the two family prototypes detect the same set of fragments in filter hybridization experiments. Since the homologies among the other family members in this subgroup are similar (Table 1), this result suggests that most if not all genes belonging to different families, can be detected with only one probe under our hybridization conditions (see Methods). The approximatively ten bands detected with the VH441 and VH283 probes, which

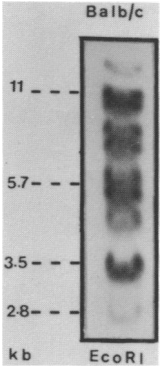

FIGURE 5 :
A genomic blot analysis of a BALB/c DNA digested by EcoRI, hybridized with either the EcoRI insert of VH441 which belongs to the antigalactan family or the PstI insert of VH283 which belongs to the anti-SRBC family, displays the same pattern. Fragment size (in kb) was estimated from EcoRI digest of recombinant phages run in parallel.

represent respectively the antigalactan and anti-SRBC families, probably include genes from the antiphosphorylcholine and anti-inulin families. Our estimation is in good agreement with the prediction of Rabbitts et al. that 10-15 VHIII genes are present in the mouse genome (13).

In Figure 6, it is shown that the homology among four members of the

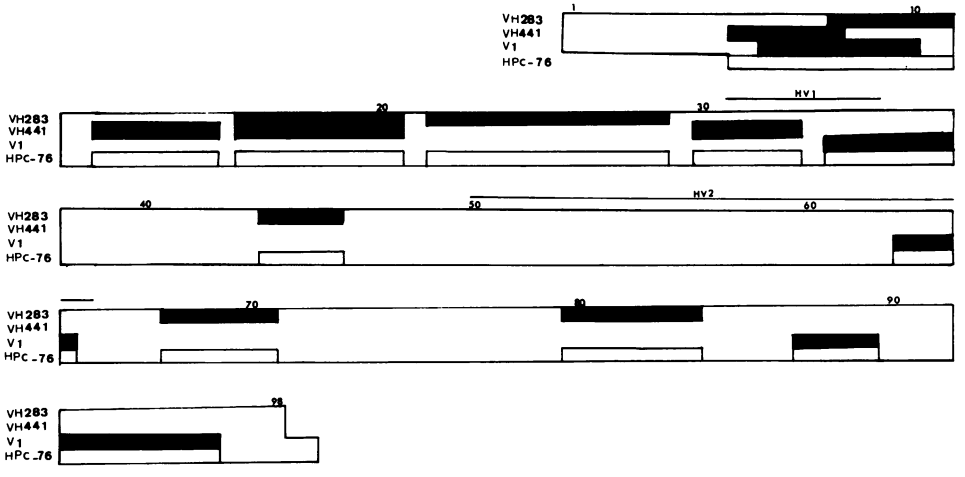

FIGURE 6 : A diagrammatic representation of homologous stretches between HPC-76 and the three other mouse VH from different families of VHIII subgroup illustrates that HPC-76 could be reconstructed by combinations of segments from these other genes.
It is worth noting that the choice of HPC-76 to amplify this homology is arbitrary and also the choice of the minimal length of nucleotides held for diagram. Solid boxes represent stretches (8 to 22 bp - see Fig. 4 for nucleotide sequence) homologous to open boxes on HPC-76 gene.

VHIII subgroup consists of stretches of 8 to 22 consecutive nucleotides. This comparison reveals that the VHPC-76 anti-inulin gene, which shares about 71-75% homology with the three other VH genes, could be reconstructed for most of its sequence including the first hypervariable region (HV1) by assorting different gene segments derived from the three other families. Two observations are of particular interest concerning the HV regions. First, there is a sequence of ten homologous nucleotides surrounded by two divergent nucleotides on both sides of the HV of VH441 and VHPC-76 germ-line genes. Secondly, at the end of the HV2 region is observed a stretch of 16 nucleotides, which are identical except for two adjacent codon deletions in VH283 and V1. These segments are otherwise immediately flanked by a very divergent sequence.

Similar observations have been previously made by Kabat based on comparison of amino acid sequences.

The patterns of segmental homology described in this paper, which comprise stretches of identical nucleotides, strongly suggest that these gene families have been established by recombination between more divergent ancestral genes. The hypervariable region HV2 seems to be less affected by these putative recombinational events. Segmental homology between linked genes has been explained by gene conversion (18,19,20,21,22,23). This mechanism is a powerful means of generating paradoxical homogenization and polymorphism in a multigenic family and consequently of providing advantageous combinations for the repertoire. Stretch homology and multiplicity of VH genes may be considered the footprints of such events. The establishment of these four families occurred before mammalian radiation because the mouse VH283 and human VH11 described by Rechavi et al. (4) are 82,5% homologous, while the homology between the VHIII gene families is around 71-75%. Particular combinations of antibody gene segments appear to be strongly conserved after mammalian radiation judging by the similarity of the human and mouse VH anti-SRBC.

The finding of a large number of human SRBC V genes demonstrates that amplification or contraction might have taken place later in the evolution of different species. The possible selective pressure on VHIII genes to preserve an evolutionary successful subgroup leads to the paradox that these variable region genes in man and mouse are more conserved than the corresponding constant region genes $C\kappa$, which have only 63,7% homology. Thus, in spite of their potential to diversify rapidly by somatic mutation, VH genes appear strongly conserved during evolution.

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