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Length Distribution of CDRH3 in Antibodies

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ABSTRACT **Sequences of the third complementarity determining region of antibody heavy chains (CDRH3s) are listed according to their length. Human sequences vary from 2 to 26 amino acids residues, but less extensively in other species. When combined with the other five complementarity determining regions, this enormous length variation of CDRH3, together with amino acid substitutions in their sequences, can provide a very large number of antibody specificities and can influence the shape of antibody combining sites.**

Key words: heavy chains, complementarity determining region, antibody specificity, amino acid loops, V-(D)-J joining

INTRODUCTION

Complementarity determining regions (CDRs) of light and heavy chains form the antibody combining site.[1–3] Among these six short segments, the third CDR of the heavy chain (CDRH3) varies most extensively in length. It can be as short as two amino acid residues, while the longest known one has 26 residues.[3] Functionally, CDRH3 plays a distinct role in determining antibody specificity.[4–12] The other five CDRs, as most strikingly shown from the high resolution crystallographic structures of lysozyme–antilysozyme complexes[5,13,14] (for stereophotos of antibodies and antigen–antibody complexes as drawn from alpha-carbon coordinates, see ref. 3), also form part of the antibody combining site and contribute to the total binding energy.

The present study lists all complete CDRH3 amino acid sequences in various species together with their antibody specificities if known. The length distribution of these CDRH3 sequences seems to be distinct for each species although the currently available sequences are not randomly selected. This collection of data may also be useful for learning how antibody specificities are related to sequences and for designing antibodies with required specificities by choosing the CDRH3 sequences associated with that specificity.

MATERIALS AND METHODS

Published amino acid sequences of immunoglobulins have been collected and aligned so that their CDRH3s can be identified precisely.[3] Most of them are listed in ref. 3, and others are stored in the NIH supported PROPHET computer system.[15] They vary in length and are positioned from 95 to 102 with possible insertions 100A to 100K. Some of the CDRH3s are longer than 19 amino acid residues. These sequences are also aligned to the above positions with # to indicate additional inserted residues. Dashes are introduced for alignment, numbered 100A–100K and placed before residue 101.[3] For an alternative alignment to show V-D-J joining, see Wang et al.[16] The IUB-IUPAC single letter codes are used to identify amino acid residues.

For each species, CDRH3s are listed in the order of decreasing length in tables available on request.* The name of the immunoglobulin is listed first, followed by the amino acid sequence, the antibody specificity, if known, and the reference. If a sequence is found in several immunoglobulins, its names are listed together. If different antibody specificities are associated with the same sequence, they are also listed together. Sequences of the same length are alphabetized by their single letter amino acid code.

RESULTS

Length distributions of CDRH3 are listed in Table I for human, mouse, rabbit, other and all species.

*Send a 3.5- or 5-inch diskette and computer architecture (i.e., IBM, Macintosh) and a prepaid Federal Express order.

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TABLE I. Length Distribution of CDRH3 Sequences in Human, Mouse, Rabbit, Other and All Species*

	Length distribution of CDRH3 in various species				
Amino acids	Human	Mouse	Rabbit	Other species	All species
19+	14 (7)†	0 (0)	0 (0)	1 (0)‡	15 (7)
19	7 (2)	1 (1)	4 (0)	0 (0)	12 (3)
18	6 (3)	1 (1)	2 (0)	0 (0)	9 (4)
17	10 (6)	5 (3)	0 (0)	0 (0)	15 (9)
16	10 (6)	12 (8)	5 (0)	0 (0)	27 (14)
15	8 (1)	14 (10)	7 (0)	1 (1)	30 (12)
14	17 (9)	33 (21)	5 (1)	2 (0)	57 (31)
13	20 (6)	40 (35)	12 (1)	2 (0)	74 (42)
12	15 (6)	128 (82)	8 (0)	1 (0)	152 (88)
11	17 (6)	115 (80)	7 (1)	6 (0)	145 (87)
10	21 (7)	138 (87)	8 (0)	5 (1)	172 (95)
9	8 (5)	155 (100)	4 (0)	11 (1)	178 (106)
8	12 (5)	130 (79)	3 (0)	4 (0)	149 (84)
7	5 (1)	89 (56)	3 (1)	1 (0)	98 (58)
6	2 (0)	35 (16)	1 (0)	1 (0)	39 (16)
5	2 (2)	64 (43)	0 (0)	2 (0)	68 (45)
4	1 (0)	28 (9)	1 (0)	0 (0)	30 (9)
3	1 (0)	11 (3)	0 (0)	0 (0)	12 (3)
2	1 (0)	5 (1)	0 (0)	0 (0)	6 (1)
Total	177 (72)	1004 (635)	70 (4)	37 (3)	1288 (714)

*For each length, the total number of known sequences is listed. The numbers in parentheses indicate sequences with known antibody specificities.
†Numbers in parentheses indicate the number of those with known specificities. There are 5 (2) sequences with 20 residues, 2 (1) with 21, 1 (1) with 22, 1 (1) with 23, 2 (0) with 24, and 3 (2) with 26.
‡Chicken HC86 has 24 residues.

The numbers in parentheses denote sequences with known antibody specificities. For human and mouse, for which many CDRH3 sequences have been determined, 41 and 63% have known specificities, respectively. These distributions are also plotted in Figures 1–4 in which the lower stippled portions indicate the numbers of sequences with known specificities, and the entire bars indicate the total number of sequences.

For human CDRH3, the length varies from two to 26 amino acid residues, with a central peak from 10 to 14 residues and lesser peaks at 8 to 9 and 15 to 19 residues (Fig. 1). In the mouse, for which much more data are available, the distribution is more restricted, with none longer than 19 amino acid residues. There is a central peak from 8 to 12 residues (Fig. 2). For rabbit, numbers of known CDRH3 sequences are much fewer. They vary in length from 4 to 19 (Fig. 3). In other species, there is one chicken sequence having 24 residues, while the remaining vary from 5 to 15 residues. The length distribution for all species is shown in Figure 4, and is dominated by the large number of mouse sequences.

DISCUSSION

For human, three of the longest CDRH3s have 26 amino acid residues: Ab18 is a polyreactive autoantibody, 783c has no known specificity, and KIM36H is anti-DNA. There are 14 sequences longer than 19 residues, seven of which have known specificities: three rheumatoid factors, two autoantibodies, one anti-DNA, and one anti-cytomegalovirus. Among human antibodies with known specificities, many are rheumatoid factors and autoantibodies suggesting that this collection may be nonrandom. The shortest known human CDRH3 consists of only two residues. As shown in Table I and Figure 1, the length distribution of human CDRH3 shows a peak at 10 to 14 with lesser peaks at 8 to 9 and 15 to 19 residues. Chothia and Lesk[6] first tabulated length variations of 54 human and mouse CDRH3s and noted that the preferred lengths were 8 and 12 residues. Their definition of CDRH3 was two residues shorter, so that they listed 6 and 10 residues instead. However, our data can be fitted with a Poisson distribution with mean length of 11.6 residues and normalized least square value of 0.00577. Thus, there is no indication that length distribution is significantly different from random. This seems to be in agreement with the result of Yamada et al.[17] on polymerase chain reaction amplified CDRH3 segments from human peripheral blood B lymphocytes. Human CDRH3s with known specificities have a similar distribution.

Length distribution of mouse CDRH3s is somewhat different. Even though the sample size is about

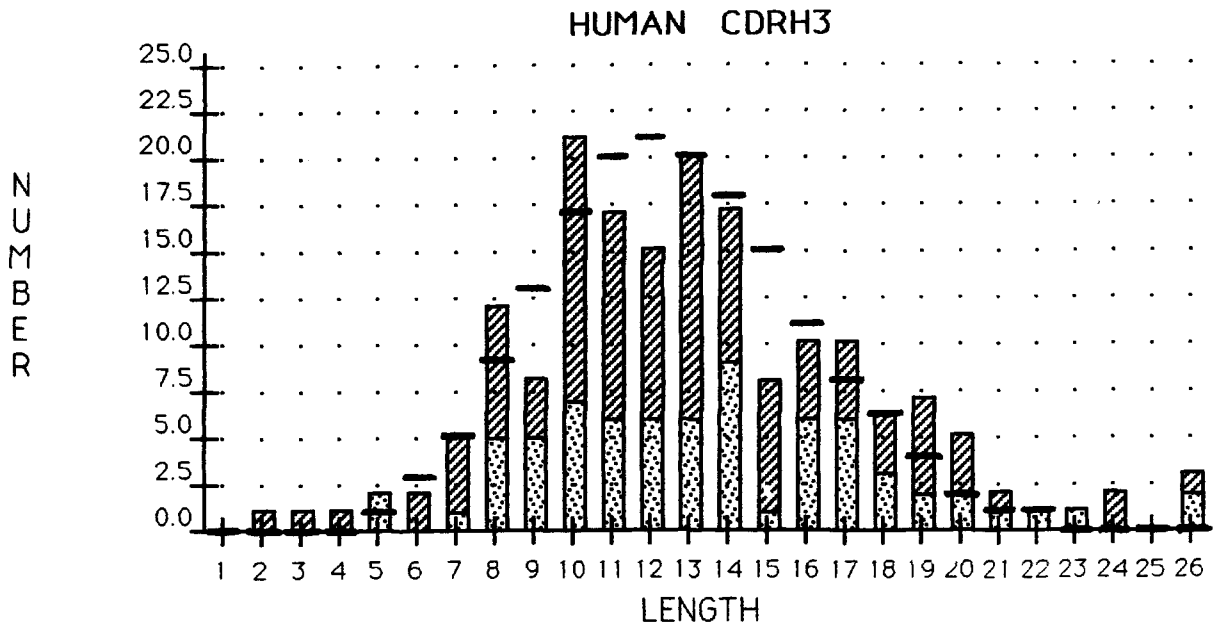

Fig. 1. Length distribution plot for CDRH3 sequences from human. The bottom stippled portions indicate the numbers of sequences with known antibody specificities. Entire bars indicate the total numbers of complete sequences. A Poisson distribution with mean length of 11.6 residues is indicated by horizontal lines.

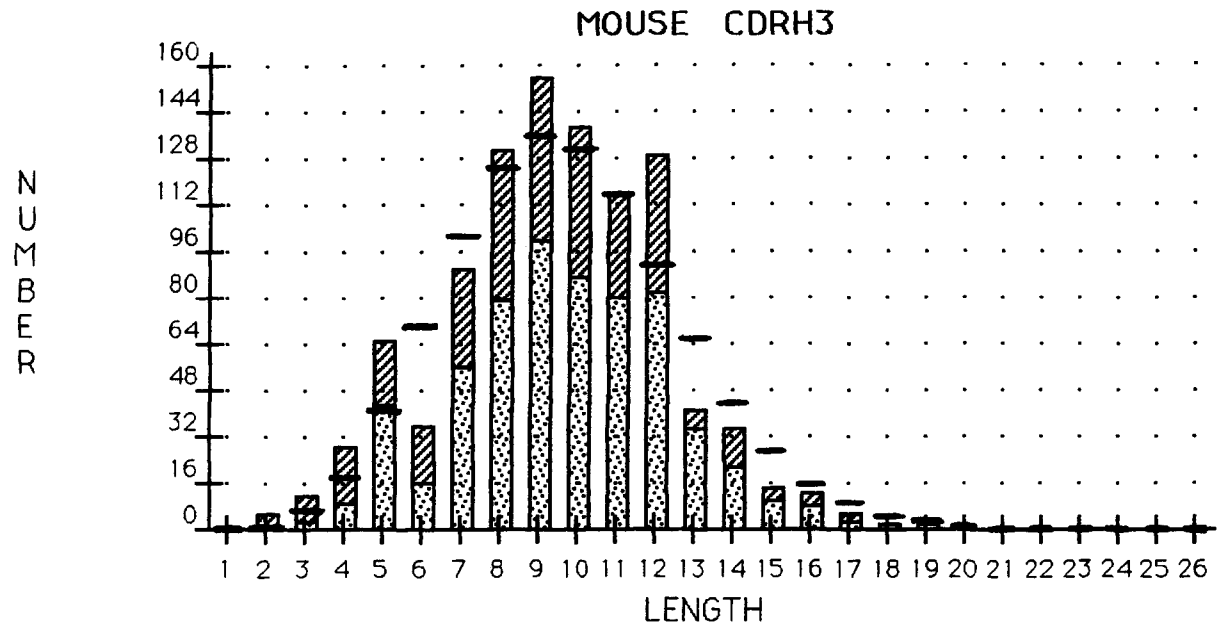

Fig. 2. Length distribution plot for CDRH3 sequences from mouse. The bottom stippled portions indicate the numbers of sequences with known antibody specificities. Entire bars indicate total numbers of complete sequences. A Poisson distribution with mean length of 8.7 residues is indicated by horizontal lines.

six times larger than the human sample, there is only one with 18 and one with 19 residues and none larger, suggesting that mouse antibodies might be derived from more restricted populations. As shown in Table I and Figure 2, length distributions of mouse sequences with and without known specificities are centered around a peak of 7 to 12 residues. Again, the data (Fig. 2) are close to a Poisson distribution of mean length of 8.7 residues with a normalized least square value of 0.00481. Thus, on the average, mouse CDRH3s are three amino acid residues shorter than human CDRH3s. This might be due to the existence of relatively longer D-minigenes in human.[3] The shortest mouse CDRH3s also have just

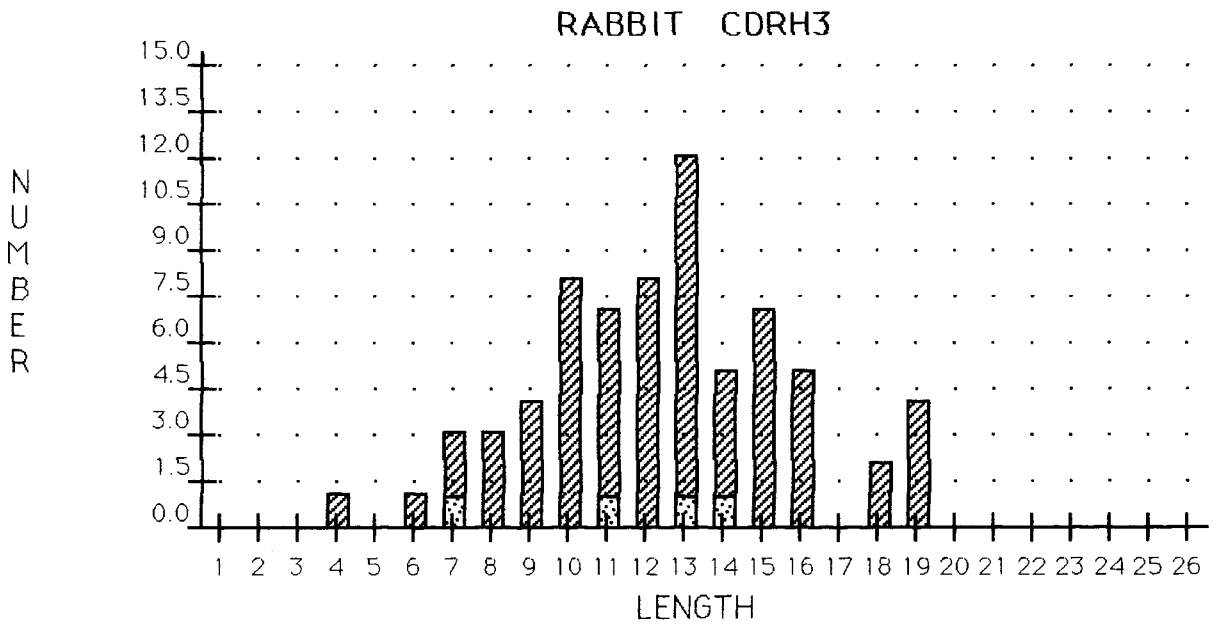

Fig. 3. Length distribution plot for CDRH3 sequences from rabbit. The bottom stippled portions indicate the numbers of sequences with known antibody specificities. Entire bars indicate total numbers of complete sequences.

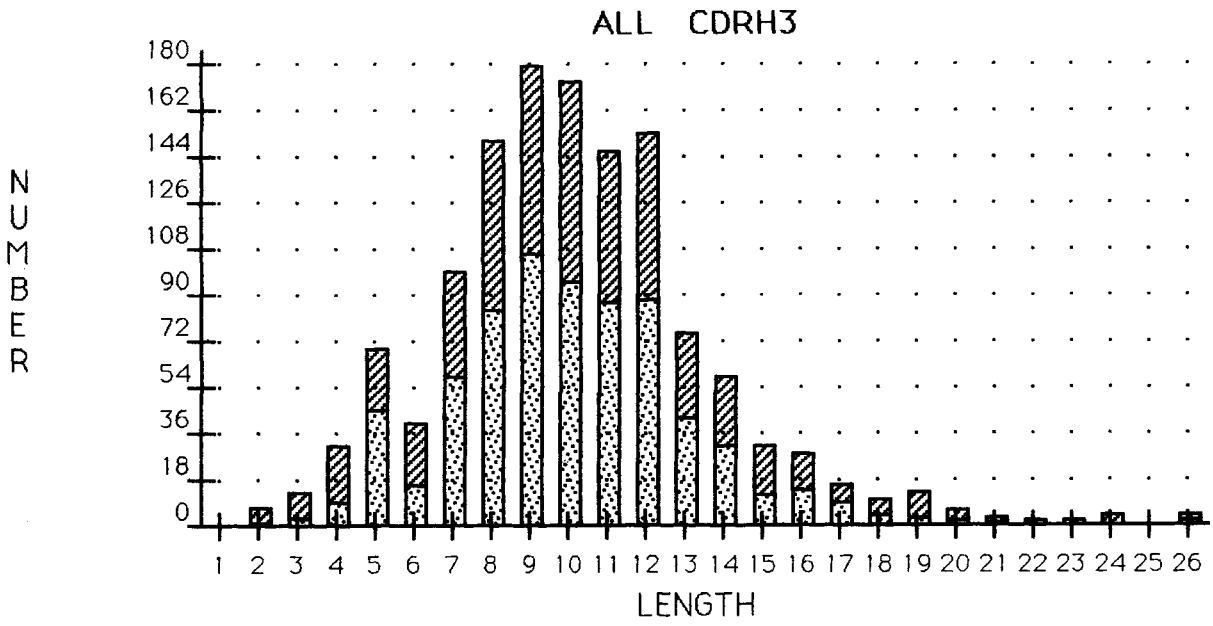

Fig. 4. Length distribution plot for CDRH3 sequences from all species. The bottom stippled portions indicate the numbers of sequences with known antibody specificities. Entire bars indicate total numbers of complete sequences.

two residues which can be derived completely from the J-minigenes so that no D-segment is present.[18–20] Lewis et al.[21] proposed that in heavy chains such V-J joining without D can be explained by hybrid joint formation, a variant of the "standard" 12/23 signal joining. The rabbit CDRH3 sample is much smaller and its length distribution is slightly more restricted than that of the mouse (Table I and Fig. 3).

For other species, sample sizes are even smaller and their sequences are listed together. Except for a chicken CDRH3 with 24 residues, all others are between 15 to 5 residues in length. When more sequences become available, some of these can be

listed separately for each species. For *Xenopus*,[22] the length distribution of CDRH3s in adults is similar to that of mouse, but froglet and tadpole CDRH3s are much shorter (E. Hsu, personal communication). Due to the large number of mouse sequences, the length distribution of CDRH3 for all species (Table I and Fig. 4) behaves like that of mouse.

To study the effect of CDRH3 length on binding of antigen by antibody, three antilysozyme–lysozyme complexes[5,13,14] have been analyzed in detail.[11] CDRH3 lengths of these antibodies against the same antigen are different: eight residues for D1.3, seven for HYHEL-5, and five for HYHEL-10. They are specific for different epitopes of chicken egg-white lysozyme. As the length of CDRH3 shortens, the antibody may accommodate other segments of the lysozyme molecule which may become accessible with other CDRs playing more important roles in binding the antigen. Indeed, CDRH3s of D1.3, HYHEL-5, and HYHEL-10 are in contact with 10, 4, and 3 amino acid residues of lysozyme, respectively, i.e., directly related to their lengths.[5,11,13,14] On the other hand, for shorter CDRH3s, both CDRH1 and CDRH2 become more involved. CDRH1s of D1.3, HYHEL-5, and HYHEL-10 are in contact with two, two, and seven residues of lysozyme, and CDRH2s with three, six, and seven residues, respectively,[5,11,13,14] i.e. inversely related to their CDRH3 lengths. When more 3-D antigen–antibody complex structures are determined, the above findings can be further substantiated.

CDRH3s of eight to nine amino acid residues occur frequently especially in mouse. As exemplified by the detailed 3-D structure of antilysozyme D1.3[5] the antibody combining site with a CDRH3 of eight residues appears as a rather flat surface with side chain depressions and protrusions in perfect complementarity to the surface of the lysozyme molecule. E225[23] with an eight-residue CDRH3 and NEW[24,25] with a nine-residue CDRH3 also show relatively flat combining sites. In the stereo-pictures of α-carbon 3-D structures of antibody combining sites in the Introduction of ref. 3, all CDRH3s appear as loops which may be the preferred structure.[26] Other antibodies with eight residues (YST9-1, Jel318) and nine residues (J539, SE155-4) have CDRH3 loops more or less even with the loops of other CDRs, suggesting that their combining sites might also be somewhat flat. Those with fewer residues, POT (four), HYHEL-10 and NQ10/12.5 (five), AN02 (six), and MCG, HYHEL-5, 4.4.20, and HED10 (seven) have slightly recessed CDRH3 loops. Alzari et al.[27] have compared the combining site of NQ10/12.5 with that of D1.3, and found that a cavity on the NQ10/12.5 site due to a shorter CDRH3 of five residues was filled by the hapten 2-phenyloxazolone, while the same space in the D1.3 site was occupied by the longer CDRH3 of eight residues. On the other hand, those with more residues, BV0401 and B13I2 (10), NC41 and MCPC603 (11), 36–71 and HIL (12), Jel72 (13), R19.9 (15), and KOL and 3D6 (17), show somewhat protruded CDRH3 loops. The monoclonal antibody R19.9 specific for the *p*-azobenzenearsonate group[28] has a 15-residue CDRH3 which forms a long loop protruding into the solvent. The 17-residue CDRH3 of KOL has a slightly different structure.[28,29]

Extensive variations of middle amino acid residues as well as residues at the N-terminal end of mouse CDRH3s suggest that these positions may be more important in conferring specificity than the two C-terminal end residues which are frequently DY and might be structural; DY is derived from the J_H2 and J_H4 minigene sequences in the mouse and J_H4 minigene in human. When associated with the same specificity, CDRH3s also show amino acid variations in the middle positions. For example, the 15 mouse anti-(4-hydroxy-3-nitrophenyl)acetyl CDRH3s with nine residues have YYYG at the N-terminal end, and DY in 13 sequences at the C-terminal end. These antibodies may bind the hapten molecule in different orientations since their middle three residues vary extensively. On the other hand, the binding of hapten may be due to the conserved sequence YYYG at the N-terminal of CDRH3. Indeed, several other anti-(4-hydroxy-3-nitrophenyl)acetyl antibodies with various lengths of CDRH3 contain YYY or YYG in their sequences.

In general, for antibodies with the same specificity, lengths of their CDRH3s can vary. For example, anti-(4-hydroxy-3-nitrophenyl)acetyl antibodies can have CDRH3s ranging from 4 to 15 residues. These antibodies may be directed toward different epitopes of the same antigen. On the other hand, as previously noted,[11] there are two CDRH3 sequences, each of which is associated with two different specificities. One of these YSNYWYFDV, is found in anti-bromelain-treated mouse red blood cell and anti-*E. coli* antibodies. However, both antigens may share the same phosphatidylcholine epitope[11] so they may not have different specificities. The other, LHYYGYAAY, occurs in anti-β-1,6-D-galactan and anti-β-2,6-fructosan antibodies.[11] Both CDRH3s are nine residues in length.

The vast data of mouse CDRH3 sequences against many different antigens can be used to design antibodies of different specificities.[30] However, restrictions on their length from two to 19 residues does not indicate that longer CDRH3s should be excluded from consideration. As shown in the tables available on request, several human CDRH3s are longer and are associated with known specificities.

Since CDRH3 is located in the middle of the antibody combining site, its length may play an important role in fitting the contour of the antigen molecule (see stereophotos of α-carbon 3-D structures in the Introduction of ref. 3). For a short CDRH3, the

antigen surface might protrude into the depression not filled by CDRH3.[27] On the other hand, long CDRH3s might extend out of the combining site and interact with recessed portions of antigens.[28]

Another aspect of CDRH3 sequence variation is the positioning of identical amino acid segments at varying locations in CDRH3. For example, the tetrapeptide YYGS is found in many different human and mouse CDRH3s at positions 95 to 98, 96 to 99, 97 to 100, 98 to 100A, 99 to 100B, or 100 to 100C. The resulting CDRH3s are associated with antibodies of numerous specificities: anti-DNA, anti-IgG, anti-(4-hydroxyl-3-nitrophenyl)acetyl, anti-phosphatidylcholine, anti-mAb HP-Id22, anti-α-1,6-dextran, anti-(T,G)-A—L, anti-CD7, anti-dinitrophenyl, anti-phosphorylcholine, anti-influenza virus hemagglutinin, anti-5-dimethylaminonaphthalene-1-sulfonyl, anti-trinitrophenyl, anti-β-1,6-galactan, anti-poly(Glu-60,Ala-30,Try-10), anti-phenyloxazolone, plus 21 sequences of unidentified specificities. Another tetrapeptide, SSGY, occurs in CDRH3 of human, mouse, rabbit, and channel catfish, at positions 100B to 100E, 99 to 100B, 95 to 98, and 97 to 100 respectively. These findings contribute substantial additional diversity to CDRH3 and make possible loops with different relationships and lengths to V_H genes, to N or P segments,[31,32] and to J_H-minigenes. Both YYGS and SSGY are represented in germline D-minigenes.[3]

CDRH3s can vary in length as well as in amino acid sequence. This implies that there are at least two mechanisms for CDRH3 to confer fine specificity. Thus, unlike the other five CDRs which have one or a small set of main-chain conformations,[6,7] CDRH3s have no canonical structures.[4–14] This extensive variation of CDRH3 is due to the well known processes of combining D- and J-minigenes[33–35] with the possible addition of N or P segments.[31,32] Together with the other five CDRs, whose length variations are relatively limited, a species can easily generate a large repertoire of different antibody specificities.

Our present listing of CDRH3 sequences should be updated continuously as new heavy chain variable region sequences of immunoglobulins, especially those with known specificities, are determined. These data might provide a basic understanding of the mechanisms of CDRH3 conferring fine antibody specificity as well as a starting point for the design of antibodies with desired specificities. The T-cell receptor data should be examined in a similar manner.

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