Structure, Diversity, and Repertoire of V<sub>H</sub> Families in the Mexican Axolotl

Rachel Golub and Jacques Charlemagne

*J Immunol* 1998; 160:1233-1239;  
http://www.jimmunol.org/content/160/3/1233

References  
This article cites 47 articles, 7 of which you can access for free at:  
http://www.jimmunol.org/content/160/3/1233.full#ref-list-1

Subscription  
Information about subscribing to *The Journal of Immunology* is online at:  
http://jimmunol.org/subscription

Permissions  
Submit copyright permission requests at:  
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts  
Receive free email-alerts when new articles cite this article. Sign up at:  
http://jimmunol.org/alerts
The V region of the Ig heavy (H)\(^3\) chain of jawed vertebrates is generated by the random assembly of multiple V, D, and J germline-encoded segments (1). This combinatorial diversity is greatly enhanced by such somatic events as the deletion or addition of nucleotides at the V-D and D-J junctions, point mutations, gene conversion, or editing. The germline repertoires of the V\(_H\) segments have been studied, and the V\(_H\) sequences that have 75% or more identical amino acids in a given species are assigned to the same V\(_H\) family. This classification generally agrees with classifications based on the similarities of the nucleic acid sequences. These are determined using V\(_H\) DNA probes on genomic Southern blots with a washing stringency that allows specific VH probes showed restriction fragments from 1 (V\(_H\) 9) to 11–19 (V\(_H\) 2), and the total number of V\(_H\) genes was 44 to 70, depending on the restriction endonuclease used. The V\(_H\) segments were not randomly used by the H\(_\alpha\) and H\(_\nu\) chains; V\(_H\) I, V\(_H\) 6, and V\(_H\) 11 were underutilized; and the majority of the V\(_H\) segments belonged to the V\(_H\) 7, V\(_H\) 8, and V\(_H\) 9 families. Most of the nine J\(_H\) segments seemed to be randomly used, except J\(_H\) 6 and J\(_H\) 9, which were found only once in 79 clones. The Journal of Immunology, 1998, 160: 1233–1239.

A limited number of representative vertebrate species have been extensively analyzed. The number of V\(_H\) families varies from one species to another, and these variations do not seem to be related to phylogeny. Two V\(_H\) families have been described in the horned shark (Heterodontus francisci) and in the skate (Raja erinacea) and 1 in the nurse shark (Ginglymostoma cirratum); but 6 V\(_H\) families have recently been described in carcharine sharks, e.g., the sandbar shark (Carcharhinus plumbeus) and the bull shark (Carcharhinus leucas) (3, 4). The best-studied teleost fish species are the channel catfish (Ictalurus punctatus), with 7 V\(_H\) families, and the rainbow trout (Oncorhynchus mykiss), with 11 V\(_H\) families (5, 6). The clawed toad, Xenopus laevis, has 11 V\(_H\) families (7), and the red-eared turtle (Pseudemys scripta) has at least 4 V\(_H\) families (8). A single functional V\(_H\) gene has been found in the chicken, which becomes diversified somatically by the conversion of stretches of V\(_H\) nucleotides provides by numerous nonfunctional V\(_H\) pseudogenes (9). The best-known IgH locus in mammals is the human one, which includes 64 V\(_H\) segments (31 of which being pseudogenes) that can be divided into 6 distinct families (10). The laboratory mouse has 14 V\(_H\) families, which is the most diverse organization described so far in a vertebrate (11). Some mammalian species, however, have a restricted number of V\(_H\) families. The sheep V\(_H\) repertoire is derived from a single germline gene family with about 10 members, which is related to the human V\(_H\) 4 family and the murine V\(_H\) I subgroup (12). All of the approximately 100 V\(_H\) genes of the rabbit seem to belong to the same family, homologous to the human V\(_H\) 3, but the 3’-most V\(_H\) 1 gene is used preferentially and somatically diversified by gene conversion (13). There is also a single V\(_H\) family in the pig, and it is related to the human V\(_H\) 3 family, the rabbit V\(_H\) 1 gene, and the single functional gene of the chicken (14).

Studies on Xenopus and on the Mexican axolotl (Ambystoma mexicanum) have demonstrated that both species synthesize class IgM and IgY Ig, but that a third class, IgX, is produced only by Xenopus. The amino acid sequences of the axolotl C\(_\mu\) and C\(_\nu\) chains are remarkably similar to those of their respective Xenopus counterparts (15, 16). The IgH locus of Xenopus includes a large number of V\(_H\) segments, divided into 11 families of about 1 to 40 members (7, 17) and, although subjected to some limitation (18), the Xenopus-specific Ab repertoire approaches the avian and mammalian schemes in its complexity (17). Like the more advanced vertebrates, Xenopus synthesizes IgM class Abs during the primary response and IgG-like IgY Abs in the secondary response to thymus-dependent Ags (19). This is not so in the axolotl, where almost all specific Abs belong the IgM class (20). No typical Ab enhancement occurs after antigenic challenge, IgY Abs are not involved, and responses do not depend on thymus-derived cells,
even those to potentially thymus-dependent Ags (21–23). Furthermore, the very simple, constant isoelectric focusing (IEF) patterns of the H and L chain spectrotypes from anti-DNP Abs suggest that specific Abs use a restricted repertoire of VH and VL elements (24). Thus, although anuran and urodele amphibians are considered monophyletic and as having diverged from primitive salamander-like ancestors some 270 million years ago (25), the potential of their respective VH repertoires seem to have strongly diverged during evolution. We have recently described the cDNA transcripts of 3 VH families in the axolotl (26). The present work describes the VH repertoire of this urodele species using VH cDNA libraries obtained by anchored PCR and also examines the genomic diversity using Southern hybridization.

Materials and Methods

Construction of VH libraries

Total cytoplasmic RNA from the spleen cells of 10-mo-old unimmunized axolotls was used to build 2 VH libraries by anchored PCR using a commercial kit (5′ system for rapid amplification of cDNA ends (RACE), Life Technologies, Cergy-Pontoise, France), essentially following the manufacturer’s instructions with minor modifications (27). Briefly, first-strand cDNA was synthesized using specific downstream primers complementary to the axolotl CM1 or CY1 primers. The amplified VH transcripts of 3 VH families in the axolotl (26). The present work describes the VH repertoire of this urodele species using VH cDNA libraries obtained by anchored PCR and also examines the genomic diversity using Southern hybridization.

Results

Definition of 11 axolotl VH families

A total of 19 independent consensus L-VH nucleotide sequences were obtained by analyzing 38 cDNA clones selected from the VH library and 41 clones selected from the VL library (all of the clones had different sequences). The amino acid sequences deduced from these clones are shown in Figure 1, together with 3 of the previously described VH sequences (26). Table III summarizes the VH families, their 41 clones selected from the VH library and 41 clones selected from the VL library (all of the clones had different sequences). The amino acid sequences deduced from these clones are shown in Figure 1, together with 3 of the previously described VH sequences (26). Table III summarizes the VH families, their

Genomic DNA was isolated from erythrocytes obtained from a single axolotl. Cells were lysed in a 1% SDS lysis buffer (50 mM Tris, pH 7.5, 100 mM EDTA) for 45 min at 65°C and digested with proteinase K (100 mg/ml) for 24 h at 45°C. DNA was isolated as described (28). The DNA (20 µg) was digested overnight with 320 U of restriction enzymes, BamHI, BglII, EcoRI, and EcoRV, and electrophoresed in a 0.8% agarose gel in TAE buffer (40 mM Tris pH 8.0, 1 mM EDTA). DNA was deproteinized by soaking the gel in 0.25 M HCl for 10 min and then denatured in 0.4 M NaOH for 15 min. DNA was transferred onto Zeta Probe membranes (Bio-Rad Iury sur Seine, France) by DNA capillary transfer for 6 h in alkaline solution (0.4 M NaOH). The membrane was briefly washed in 2× SSC and UV cross-linked. The membrane was incubated for 4 to 6 h at 65°C in a prehybridization solution (1.5× SSC, 1× SDS, 10 mM dextran sulfate, 0.5% tetrasodium pyrophosphate). DNA-homologous probes were prepared by PCR using 5′ primers specific for the FR3 regions and 3′ primers complementary to the FR3 regions of each VH family (data not shown). Fifty nanograms of DNA were labeled with α[32P]ATP by random hexanucleotide priming to 10^6 cpm/µg. Hybridization was performed for 16 to 24 h at 65°C in hybridization solution (1.5× SSC, 1× SDS, 10 mM dextran sulfate, 0.5% tetrasodium pyrophosphate, 0.5% blotto, and 5 mg denatured sonicated Escherichia coli DNA). Membranes were then washed in 4× SSC, 0.05% SDS at 65°C for 15 min, and in 2× SSC, 0.025% SDS at 65°C for 15 min, and then autoradiographed for 2 to 6 days at 80°C.

Even those to potentially thymus-dependent Ags (21–23). Furthermore, the very simple, constant isoelectric focusing (IEF) patterns of the H and L chain spectrotypes from anti-DNP Abs suggest that specific Abs use a restricted repertoire of VH and VL elements (24). Thus, although anuran and urodele amphibians are considered monophyletic and as having diverged from primitive salamander-like ancestors some 270 million years ago (25), the potential of their respective VH repertoires seem to have strongly diverged during evolution. We have recently described the cDNA transcripts of 3 VH families in the axolotl (26). The present work describes the VH repertoire of this urodele species using VH cDNA libraries obtained by anchored PCR and also examines the genomic diversity using Southern hybridization.

Materials and Methods

Construction of VH libraries

Total cytoplasmic RNA from the spleen cells of 10-mo-old unimmunized axolotls was used to build 2 VH libraries by anchored PCR using a commercial kit (5′ system for rapid amplification of cDNA ends (RACE), Life Technologies, Cergy-Pontoise, France), essentially following the manufacturer’s instructions with minor modifications (27). Briefly, first-strand cDNA was synthesized using specific downstream primers complementary to the axolotl CM1 or CY1 primers. The amplified VH transcripts of 3 VH families in the axolotl (26). The present work describes the VH repertoire of this urodele species using VH cDNA libraries obtained by anchored PCR and also examines the genomic diversity using Southern hybridization.

Results

Definition of 11 axolotl VH families

A total of 19 independent consensus L-VH nucleotide sequences were obtained by analyzing 38 cDNA clones selected from the VH library and 41 clones selected from the VL library (all of the clones had different sequences). The amino acid sequences deduced from these clones are shown in Figure 1, together with 3 of the previously described VH sequences (26). Table III summarizes the VH families, their
VH, including Cys22 (FR1), Cys92 (FR3), Trp36 (FR2), and Trp47 (FR2) (Fig. 1). Several structurally important residues were frequently present, like Gln1, Pro14, Gly44, Arg66, Tyr/Phe90, Tyr91, Ala93, and Arg94. The last five residues form the YYCAR stretch, which is present in most vertebrate VH sequences. Some uncommon residues were present. VH1, VH4, VH6, and VH10 has a Ser (or Thr) residue at position 26 (FR1) instead of the Gly, which is always found at this position in mammals. Extra Trp residues were found at positions 10 (FR1) of VH4, 14 (FR1) of VH10, 38 (FR2) of VH8, 42 (FR2) of VH11, and 52 (CDR2) of VH4. Extra Cys residues were found at position 5 (FR1) of VH10, in the CDR1 region of VH8 (3 consecutive Cys), at position 52 (CDR2) of VH8 and VH11, and position 67 (FR3) of VH4. Cys residues are also present in the CDR1 of Xenopus VH3 and the rainbow trout VH3 and VH10 segments; at position 52 (CDR2) of rainbow trout VH3 and VH10, and at position 67 of the Xenopus VH49 segment (6, 7). Finally, the Pro residues found in the CDR1 region of the axolotl VH6 and VH7 segments have no equivalent in other vertebrate CDR1.

**Genomic analysis of the VH segment repertoire**

DNA sequences representative of the 11 axolotl VH families were used to probe restriction endonuclease-digested DNA obtained from a single animal, and interpretable Southern blots hybridization patterns were obtained for each of the probes. Most of these probes revealed multiple hybridizing fragments, and there appeared to be little similarity between the hybridization patterns revealed by the various probes (Fig. 2). The number of restriction fragments ranged from 1 for VH1 and VH9 to 11 to 19 for VH2 (Table I). The total number of VH genes, estimated by adding the number of hybridizing fragments from each family, was 44 to 70, depending on the restriction endonuclease used. However, this number may be underestimated for several reasons (see Discussion).

**VHμ and VHν repertoires**

The VH and JH segments usage of the 38 VHμ and 41 VHν clones is shown in Table II. The different VH segments were not randomly used. VH1, VH6, and VH11 were clearly underutilized (only one VH6 and one VH11 segment were found among the 79 clones analyzed), and VH7, VH8, and VH9 were overused. The VH4 and VH5 segments seem to be underutilized by the μ chains. Most of the 9 JH segments (Ref. 29 and R. Golub, unpublished data) seemed to be randomly used, except for JH1 and JH5, which were overused, and JH6 and JH9, which were used in only 1 of the 71 clones bearing identifiable JH (Table II). There was no clear preference in the VH/JH combinations, except that 6 of the 8 VH5/Cv clones all had JH1, but had different VDJ junctions (the 2 other VH5/Cv clones had undetermined JH segments; data not shown). There was little redundancy among the clones. However, the VHν library contained 14 clones bearing the VH2/JH5 combination, with identical VDJ junctions.

![FIGURE 2. Southern blot analysis of axolotl VH gene families. Erythrocyte DNA from a single axolotl was digested to completion with BamHI (HI), BglII (BII), EcoRI (RI), and EcoRV (RV), fractionated in agarose gels, and transferred onto nylon membranes. Membranes were probed with DNA representing 11 VH families.](http://www.jimmunol.org/)

<table>
<thead>
<tr>
<th>Table I. Complexity of the axolotl VH families</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH Family</td>
</tr>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH1</td>
</tr>
<tr>
<td>VH1</td>
</tr>
</tbody>
</table>

*Estimated number of VH genes within each family is based on the number of hybridizing BamHI (HI), BglII (BII), EcoRI (RI), and EcoRV (RV) fragments detected by Southern blot (Fig. 2), corrected by the visual examination of the radioautographs at different exposure times. |

*An enzyme cutting site is present in the corresponding VH segment. |

*—, not done. |

*Clone 103M is identical to clone VH9 (85M).
Analysis of the CDR/H regions

Most of the mouse and human CDR1 regions, as defined by Kabat et al. (30), are 5 amino acids long, except for some V_H families (human V_H2 and V_H6, mouse V_H3, V_H8, and V_H12), which may have up to seven residues. The axolotl CDR1 regions, as defined following the same criteria, varied from three (V_H 6) to nine (V_H 2 and V_H 4) residues. Ag-interactive sites in V_H segments can also be assessed by analysis of Ig structures determined by x-ray crystallography. CDR1 can be treated as a part of a single region covering residues 26 to 35; the part of this region that forms a hairpin loop outside of the framework \( b \)-sheet spans residues 26 to 32 (region H1). In the same way, residues 52 to 56 of the CDR2 region (50 – 65) form hairpin loop H2 (31). These H1 and H2 hypervariable regions form a small repertoire of conformational canonical structures in mammals (32), and it was recently shown that the VH segments of cartilaginous fish have H1 and H2 sequences that fit well with those commonly found in the human and mouse (3). A systematic comparison of the presumed axolotl H1 and H2 sequences with the mammalian canonical structures showed that the V_H1 family segment had a three-residue H2 region (Tyr - Ser - Gly) that is identical to the three-residue hairpin that formed the H2 loop of the HyHEL-10 molecule (32) and is associated, as in HyHEL-10, to an arginine residue at position 71. The axolotl VH 7 H1 loop presents a stretch of seven residues at position 26 to 32 (Gly - Phe - Ser - Phe - Glu - Asp - Tyr) that is very similar to the H1 regions of the KOL and NEWM molecule (33) and may adopt an equivalent conformation, considering that residue Met and Arg are also conserved. For the other V_H families, the presumed H1 and H2 regions present no significant similarities with the mammalian canonical structures, although some isolated residues were well conserved (data not shown).

It has been suggested that the IgV genes have evolved so that their DNA sequence favors somatic mutation in those parts of the V segments in which the mutations are likely to affect Ag binding (34). Thus, the choice of triplets encoding the ser residue is significantly biased in the VH and V_K CDRs of several species, because AGC/T (AGY) instead of TCN falls within the intrinsic hypermutation hot spot consensus (Pu-G-Py-A/T) (35). The distributions of AGY and TCN triplets in axolotl VH genes showed a clear preference for AGY in CDR1/H1 (TCN/AGY \( \approx \) 0.73), a significant bias for TCN in FR1 (TCN/AGY = 8.25), a slight bias for TCN in CDR2/H2 (TCN/AGY = 2.71), and no significant bias in FR3 (TCN/AGY = 1.66). There was no Ser residue in the axolotl FR2 regions (Fig. 3).

Phylogenic analysis

The amino acid sequences for the 11 axolotl V_H families were compared with the GenBank database using the FASTA alignment program (36). Except for V_H6, V_H10, and V_H11, no axolotl V_H had a vertebrate V_H homologue that had more than 55% identical amino acids. The most striking similarities were between axolotl V_H1, V_H2, and V_H8, and the human V_H4 family and between axolotl V_H1 and the single sheep V_H family, which is homologous to the human V_H4 family (12). However, there were also significant similarities with lower vertebrate V_H, such as the rainbow trout V_H5 and V_H8, Xenopus V_H1 and V_H3, and the coelacanth and

Table II. V_H family usage in the V_H \( \mu \) and V_H \( \gamma \) libraries

<table>
<thead>
<tr>
<th>V_H</th>
<th>1 (5–6)( a )</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5 (5–6)</th>
<th>6 (1–3)</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10 (2–4)</th>
<th>11 (3–4)</th>
<th>( \Phi ) (ND)</th>
<th>( n ) ( b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu )</td>
<td>2( c )</td>
<td>J4</td>
<td>J4</td>
<td>J5</td>
<td>J5</td>
<td>J5</td>
<td>J5</td>
<td>J4</td>
<td>J4</td>
<td>J5</td>
<td>J5</td>
<td>( \Phi ) (ND)</td>
<td>38</td>
</tr>
<tr>
<td>( \nu )</td>
<td>0</td>
<td>J5(4)</td>
<td>J5</td>
<td>J1</td>
<td>J1(6)</td>
<td>J1</td>
<td>J2</td>
<td>J2(2)</td>
<td>J1</td>
<td>J1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>J5(4)</td>
<td>J4(3)</td>
<td>J5</td>
<td>J5</td>
<td>J6</td>
<td>J4(2)</td>
<td>J4</td>
<td>J2</td>
<td>J1</td>
<td>J1</td>
<td>(33)</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) Predicted numbers of V_H segments (see Table I).
\( b \) \( n \) indicates the total numbers of clones analyzed from each library (the number of clones bearing recognizable J_H segments are indicated in parentheses).
\( c \) Number of clones expressing a given V_H family.

FIGURE 3. Distribution of ser residues in the 11 aligned axolotl V_H families. FR regions and the number of residues limiting the FR and CDR/H regions are indicated above the sequences. Non-ser residues are indicated by hyphens; s and S indicate Ser residues encoded by TCN and AGY, respectively. The limits of the CDR (30) and H (33) regions and the s/S ratio are indicated under the sequences.
horned shark VHs are aligned with their closest homologues in Figure 4. FR1 and FR2 were the most conserved regions: for example, the FR1-CDR1-FR2 regions of axolotl VH1 and human VH4 were 79.1% homologous. The 14-amino acid-long FR2 regions were also very similar in some pairs: they were identical in the axolotl VH10 and in a VH segment belonging to the mouse VH4 family (data not shown), and there was a single amino acid difference between the FR2 of the axolotl VH1 and the human VH4 segments. The FR3 regions were also well conserved in some cases; the axolotl VH3 and trout VH8 segments were 80% homologous.

Discussion

The 79 cDNA clones isolated from the IgH and IgY libraries indicate that the axolotl has a relatively large number of VH segments, with 11 families and a pseudogene. All of the families seem to associate nonselectively with Cm or Cy, except for VH6, VH10, and VH11, which are infrequently expressed. The most commonly expressed VH families are VH7, VH8, and VH9. There was some evidence of nonuniform usage of VH families, but the sample was too small to determine the preferential association of some families to one or the other isotype (37). The same panel of JH segments was used in both isotypes, except for JH6, which was found in only 2 IgH clones.

It is clear from the that the axolotl μ and ν chains use the same collection of VH and JH segments and may have an Igk locus organized along the same lines as in mammals. However, this raises the problem of isotype switching in axolotl, where, in contrast to Xenopus, IgY are not used in the secondary responses (20), are insensitive to thymectomy (38), and can be produced by an independent population of B cells in the developing animals (38). Abundant IgY molecules are found in the stomach and intestinal epithelia of young axolotls, and they can be secreted into the gut lumen in association with secretory component-like molecules (39). These IgA-like secretory IgY molecules appear in the blood late in development (7 mo), and the amounts of IgM and IgY in the blood are similar after the animals reach 10 mo. Our results indicate that the VH usage does not seem to be restricted to the μ or ν isotype and that IgY Abs may provide a natural repertoire of wide specificity in unimmunized animals. Interpretable Southern blots were obtained for each of the VH family defined from the cDNA analysis, despite the technical difficulty caused by the large size of the axolotl genome (40). Relatedly, few members were detected in each family, but the 44 to 70 potential bands detected in the Southern blots may be an underestimate of the VH repertoire for several reasons: rare VH families may not have been detected in the cDNA libraries and thus not probed in the genome, and the presence of several hybridizing VH segments in some of the labeled bands cannot be excluded. However, the repertoire could also have been overestimated because outbred axolotls were used, so that a single VH may give rise to different bands, and also because there are restriction endonuclease sites in some of the VH segments (although Table I shows that this had a little effect on the bands counted). A single hybridizing band was found for VH9 with all of the restriction enzyme used, although four cDNA sequence variants were found. Thus, the VH9 family could be represented by a single polymorphic member.

Some of the less frequently used axolotl VH segments (VH6, VH10, VH11) have species-specific amino acid sequences that are somewhat different from all the VH in the GenBank database. Conversely, VH1, VH2, and VH8 are 55 to 65% similar to the human VH4 family, the single sheep VH4-like family, and also to other vertebrate VHs such as the Xenopus IgY, and the mouse VH2 (Q52) families (Fig. 4). The similarity between the nucleotide sequences...
of axolotl V_{H}1 and human V_{H}4 (41) reaches 80% identity, which defines a V_{H} family in a given species. The FR1 to FR2 amino acid sequences of these same V_{H} segments are 79.1% homologous, but the FR3 regions are very different, although most of the amino acid residues are conservative replacements (Fig. 4). This great similarity between sequences of V_{H} families from species so phylogenetically different may have physiologic significance. The human V_{H}4 family contains about 12 members (10) and displays little polymorphism. It is preferentially used by CD5^{−} (B1) pre-B cells (42), frequently used by B ALL and B CLL lymphomas (43), and strongly associated with anti-DNA and anti-red cell (anti-I/i cold agglutinins) autoantibodies (44, 45). The serum of unimmunized axolotls contains significant amounts of natural Abs against bacterial and erythrocyte Ags, but also against DNA and horseradish peroxidase (J. Charlemagne and A. Tournefier, unpublished data). The profile of the V_{H}4 family in the serum of unimmunized axolotl should now be monitored and their participation in natural Abs analyzed.

The V_{H}1, V_{H}6, V_{H}8, and to a lesser extent, V_{H}2 axolotl FR1 regions have amino acid sequences that are similar to the consensus FR1 sequence that defines the human and murine clan II (46). Clan II-like V_{H} sequences seem to emerge early in phylogeny (Ref. 47, and T. Roman et al., manuscript in preparation); thus, a strong selective pressure seems to have operated throughout much of vertebrate evolution to preserve the structure of V_{H} segments that may play a key role in species survival. Figure 4 shows that the 14-amino acid-residue FR2 sequence is present almost intact in V_{H} segments in phylogenetically very distant species such as the rainbow trout, coelacanth, Xenopus, human, and mouse, regardless of their family or clan membership. A stretch of 4 to 7 residues from FR2 is involved in V_{H}-V_{L} contacts in mammals and is thus important for the three-dimensional structure of Ig molecules (48). This indicates that the three-dimensional configuration of the V_{H}-V_{L} contact, which is important for building the Ag-combining site, is conserved in most vertebrate Ig.

Although some of the canonical structures of the H1 and H2 hypervariable regions in sharks and mammals are similar (3), this is not always the case in the axolotl, where the size of the CDR1 regions varies more than in mammals. This could mean that the human Ig chains have a more ancestral structure than those of the axolotl, revealing the difficulty of extrapolating to a phylogeny of vertebrate V_{H} segments from the direct comparison of sequences (T. Roman et al., manuscript in preparation). Nothing is actually known about the maturation of the humoral response in the axolotl, but the lack of germinal center-like structures in the lymphoid organs, the difficulty encountered in raising specific Abs to soluble Ags, and the absence of a typical, thymus-dependent secondary response, all suggest that there is no significant maturation of the specific immune response. However, the distributions of AGY and TCN ser codons along the axolotl V_{H} segments shows a clear bias toward the mutation-sensitive AGY codons in CDR1, a significant bias toward TCN in FR1, and no Ser codons in FR2. Thus, the axolotl CDR1 region is enriched in DNA sequences representing potential hypermutation hot spots, as in mammals (34) and Xenopus (18), and this region is flanked by DNA sequences that are more resistant to point mutations. The affinity maturation of specific Abs remains modest in Xenopus, even in the presence of a thymus-dependent IgM-IgY switch (49). However, point mutations occur, and these are often located in the AGY ser codons of CDR1 and CDR2. Thus, affinity maturation in Xenopus does not seem to be limited by the availability of mutants and might be due to the lack of an effective mechanism for selecting mutants in the absence of germinal centers (18). It was shown recently that mice lacking lymphotoxin-α (LTα−/− mice) fail to develop lymph nodes, Peyer’s patches, and germinal centers (50). These mice show specific IgM responses equal to or greater than those of wild-type mice, but have impaired high affinity IgG1 production (50), and their immune response has interesting similarities to those of normal cold-blooded vertebrates. However, the defect of LTα−/− mice can be partially corrected by hyperimmunization with large doses of Ag. These mice show somatic mutations typical of affinity maturation, although the mutations are less numerous than those found in wild-type mice (51). Thus, point mutation and affinity maturation are not absolutely dependent on germinal centers in mice, and this might be also the case in cold-blooded vertebrates such as fish and amphibians, which naturally lack primary lymphoid follicles and Ag-driven germinal centers.

Acknowledgments

We thank Louis Du Pasquier (Basel Institute for Immunology) and Bénédicte Sammut (Université de Bourgogne) for useful discussion and help in performing the Southern blots, Julien Sadreddine Fellah for advice and help, and Brigitte Cuvelier and Jean Desrosiers for editing assistance.

References