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Immunology

T-cell receptor gene homologs are present in the most primitive jawed vertebrates

(*Heterodontus francisci*/polymerase chain reaction/DNA sequencing/gene organization/gene diversity)

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ABSTRACT **The phylogenetic origins of T-cell immunity and T-cell antigen receptor (TCR) genes have not been established. A PCR approach using short, minimally degenerate oligodeoxynucleotide primers complementing conserved variable region segments amplifies TCR-like products from the genomic DNA of *Heterodontus francisci* (horned shark), a representative phylogenetically primitive cartilaginous fish. One of these products has been used as a probe to screen a *Heterodontus* spleen cDNA library and a clone was identified that is most related at the nucleotide sequence and predicted peptide levels to higher vertebrate TCR β-chain genes. Genomic analyses of the TCR homologs indicate that recombining variable and joining region segments as well as constant region exons are encoded by extensive gene families, organized in the multicluster form, characteristic of both the immunoglobulin heavy- and light-chain gene loci in the cartilaginous fishes. Greater numbers of homologous products were identified when a probe complementing the putative constant region of the TCR homolog was used to screen the same cDNA library. A high degree of intergenic variation is associated with the putative variable region segments of these isolates. Direct evidence is presented for TCR-like genes, which presumably are associated with T-cell function, at the earliest stages in the phylogenetic emergence of jawed vertebrates.**

It generally is accepted that cellular immunity evolved prior to the development of inducible, antibody-based recognition. In higher vertebrates, specific cellular immune function is mediated by T-cell antigen receptors (TCRs), which are encoded by an extensively diversified family of segmented genes and are rearranged and expressed in tissue- and developmental stage-specific manners (1–3). TCR function involves recognition of antigenic peptides bound to major histocompatibility complex (MHC) class I or II molecules (2) and diversity is achieved by many, but not all, of the mechanisms used in generating B-cell immunity (4, 5). Understanding the evolution of TCRs largely has been limited to interpretation of findings regarding gene structure and organization in several mammalian, an avian (6–8), and an amphibian (9) species. Although TCR genes have not yet been identified in bony fish, their existence is suggested by the presence of distinct T-cell functions (10, 11) and both MHC I (12) and MHC II (12, 13) genes. MHC genes also have been identified in the more phylogenetically distant cartilaginous fish (14–16); however, the chronic nature of allograft rejection and lack of other demonstrable T-cell-dependent responses question the existence of a homologous form of T-cell immunity in these species (16, 17). The objective of the investigation described here was to determine whether TCRs could be identified in a modern representative of the cartilaginous fishes, the most phylogenetically primitive extant jawed vertebrates.§

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MATERIALS AND METHODS

PCR Amplification, Subcloning, and Sequencing. The sequence of a 5′ primer complementing the conserved TCR and immunoglobulin light-chain framework region 2 (FR2) WYRQ and related motifs is CCGAATTCTGGTA(TC)-C(GA)NCA. The sequence of a 3′ (A) primer complementing the conserved (YYCA) in FR3 is CGGATCCGC(GA)CA-(GA)TA(GA)TA and a 3′ (B) primer complementing the conserved (YFCA) in FR3 is CGGATCCGC(GA)CA-(GA)AA(GA)TA. PCR mixtures contained 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 10 mM Tris·HCl (pH 8.3), 0.2 mM each of the four deoxyribonucleotide triphosphates, primers at 0.1 μM, and 2.5 units of *Taq* polymerase in a 100-μl vol and were overlaid with mineral oil. The thermal cycling protocol was 1 min at 94°C, 1 min at 45°C, and 15 sec at 72°C for 30 cycles. The final cycle was extended for 10 min at 72°C. The mixture was subjected to agarose gel electrophoresis (NuSieve; FMC); appropriately sized bands were plugged with Pasteur pipets and used as templates for a second amplification under the same conditions for 25 cycles. The mixture was then ethanol precipitated, digested with *Eco*RI and *Bam*HI, electrophoresed in low melting point agarose, and cloned into M13 (mp18 and mp19).

cDNA and Genomic Library Screening. A *Heterodontus* spleen cDNA library (18) was lifted onto charged nylon filters and hybridized with the TCR homologous PCR product HFYYC182, labeled with [^{32}P]dCTP using a random nonamer method, at 65°C for 16 hr in 0.6 M NaCl/0.02 M EDTA/0.5% SDS/0.1% sodiumpyrophosphate/0.2 M Tris·HCl, pH 8.0. The filters were washed at 52°C in 1× SSC (0.15 M NaCl/0.015 M sodium citrate)/0.05% sodiumpyrophosphate/0.1% SDS and subjected to autoradiography. A 214-bp probe was generated from the putative first exon of the constant (C) region of cDNA HF6 by PCR. Approximately 0.8 haploid genome equivalent of a *Heterodontus* liver genomic DNA library in λ DASH (Stratagene) was screened with the same probe and under the same conditions described for the cDNA library. Oligonucleotides complementing the putative HF5 transmembrane (5′-TTTGCAGATGAGGATCAGGTA-3′) and cytoplasmic/3′ untranslated region (5′-GGTTTGACT-GATCGTCGCAA-3′) exons were end-labeled and used as probes for genomic clone mapping.

Abbreviations: C, constant; V, variable; J, joining; D, diversity; FR, framework region; MHC, major histocompatibility complex; TCR, T-cell antigen receptor; V_T, TCR V region; C_T, TCR C region; J_T, TCR J region.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U09531, U09532, U09533, and U09534 for genes or gene segments HF11, HF15, HF16, and HF17, respectively).

DNA Sequence Analysis. DNA sequencing of the initial PCR amplification products, cDNA, and segments of genomic clones used the dideoxynucleotide chain-termination method with ^{35}S-labeled dATP. Initial data bank searches of genomic PCR amplification products and cDNA sequence used the FASTDB program of IntelliGenetics. The following parameter settings for genomic PCR product analyses were used: similarity matrix, unitary; *K*-tuple=2; translation frame, 3; mismatch penalty=1; joining penalty=20; gap penalty=1.00; window size=32; gap size penalty=0.05; cutoff score=10. Searches were made against GenBank 79. Initial matches were then sorted by optimized score. Initial alignments of TCR C region (C_T) segments of TCR gene homologs from various species were achieved by using the GENALIGN program of IntelliGenetics and were subsequently optimized by manual inspection [GenBank accession numbers: HF6 cDNA, U07624; genomic TCR variable (V) region (V_T) segment HF2VA, U07623; genomic TCR joining (J) region (J_T) segment HF1JA, U07622].

CHEF Analysis. Agarose-embedded erythrocytes (5 μg of DNA per lane) were digested for 16 hr with 40 units of the respective enzymes. Pulse-field gels were run on a CHEF-DR II system (Bio-Rad) using a 1% Seakem LE (FMC) agarose gel in 0.05 M Tris·HCl/0.05 M boric acid/1 mM EDTA at 200 V; switch time, 60 sec for 15 hr and 90 sec for 8 hr. After visualization of standards, the gel was blotted onto Biodyne B (Pall), hybridized with random nonamer-labeled V_T- and C_T-specific probes, and exposed for 5 days. Between hybridizations, the blot was stripped and exposed to confirm complete removal of the probes.

RESULTS AND DISCUSSION

Previously, we described an immunoglobulin light-chain gene in *Heterodontus francisci*, which exhibits greater nucleotide sequence identity with certain mammalian TCR genes than with higher vertebrate light-chain genes (19). The data base of light-chain gene sequences from lower vertebrate species has been expanded recently (ref. 20; unpublished observations) and comparisons of the predicted peptide sequences of these light-chain, higher vertebrate immunoglobulin light-chain TCR, and CD8 (1, 21) genes indicate that two four-amino acid stretches in FR2 of both TCR and immunoglobulin light chain (WYRQ) and FR3 [Y(Y/F)CA] exhibit little phylogenetic variation. Specific primers complementing 11 nucleotides in each of these sequences have been synthesized; nucleotide sequence degeneracy of the 5′ (FR2) primer is 16-fold and the two different 3′ primers (A and B) are both 8-fold degenerate. The primer sets are expected to yield PCR products in the 179- to 197-bp range and the 5′ primer was designed specifically to not fully complement immunoglobulin heavy-chain genes, which typically possess the W(V/I)RQ motif. The sequences of three different 193- to 197-bp products resulting from amplification of *Heterodontus* genomic DNA in which the 3′ (A) primer was used and one product resulting from amplification in which the 3′ (B) primer was used had sequence identities at the predicted peptide level that were highest with TCRs, ranging from 30% to 45%. After subtracting the primer contributions, the products are 20–40% related to each other at the predicted peptide level and may represent different TCR classes or extensively diversified families.

One of these genomic PCR amplification products, HFYYC182, was used as a probe to screen a *Heterodontus* spleen cDNA library and multiple hybridizing plaques were detected. The complete nucleotide and predicted peptide sequences of one cDNA clone, HF6, are shown in Fig. 1. Thirty-seven of the 40 highest optimized peptide alignment scores are with TCR β-chain genes, of which the highest 34 ranking alignments are TCRs. Leader, V_T, J_T [including possible diversity (D) segment contributions], and C_T regions can be recognized by comparison to higher vertebrate TCRs. When the V_T and C_T homologous portions of cDNA HF6 were analyzed separately against the available data bases, the 50 highest scoring alignments were with the respective mammalian TCR V_β and C_β regions; maximum nucleotide identities are 57% and 56%, respectively. A sequence region that is homologous to TCR/immunoglobulin J segments is recog-

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aatcagctttgagacacaattcctgttgatatcgaagggctccaatcagttagagatttactggtttggttttgctgttgaggacaa
gtgtcgttatttagcaccgctgcagttgtgcccagcgattctgggaatcagcacatgaccccctgtgctggggcggcagcgaaatgat
cgactgtggtgtgcatcgtgagctttaccattaaatctctctctctccttattgtgtctgttctctttgacccgatctgtttctc
   ▶Leader                ▶Variable
1    M  F  L  H  S  P  V  Q  E  R  S  R  Y  P  V  G  N  R  L  T  V  A  E  G  K  T  V  E  M
     ATGTTCCTCCACAGCCCGGTCCAGGAGCGCTCTCGTTACCCAGTGGGGAACCGGCTGACGGTCGCAGAGGGGAAAACGGTGGAAATG

30   H  C  F  Q  N  D  T  S  D  S  Y  M  Y  W  Y  R  Q  Q  S  G  A  G  L  L  L  I  V  T  S
     CACTGTTTCCAGAATGACACCAGCGACAGTTACATGTACTGGTATCGCCAGCAGAGCGGAGCAGGGTTACTGCTCATAGTGACCTCG

59   I  G  T  S  D  T  S  P  E  E  G  F  K  E  R  F  K  V  T  R  P  D  L  K  T  C  S  L  K
     ATTGGCACCTCTGATACCAGCCCTGAAGAGGGTTTCAAGGAGAGATTTAAGGTCACCAGACCCGATCTTAAAACCTGCAGCCTGAAA
                                                                          ▶Joining
88   I  L  R  V  D  Q  T  D  R  A  V  Y  Y  C  A  A  S  G  H  P  S  D  S  N  S  E  A  Y  F
     ATACTGAGGGTGGATCAGACTGACAGGGCGGTGTATTACTGTGCTGCTAGCGGACACCCTTCAGACTCCAATAGCGAAGCATATTTC
                ▶Constant
117  G  D  G  T  K  L  V  V  L  G  E  N  D  T  I  R  P  A  K  V  T  V  F  E  P  S  P  E  E
     GGAGATGGGACCAAACTGGTTGTTTTAGGCGAAAACGATACGATTAGGCCTGCCAAAGTTACTGTCTTTGAGCCCTCTCCCGAAGAG

146  I  R  E  K  K  K  A  T  V  V  C  L  V  S  D  F  Y  P  D  N  I  K  I  H  W  L  V  D  G
     ATTAGAGAAAAGAAAAAAGCCACTGTGGTCTGCCTCGTCAGCGACTTCTACCCCGACAACATCAAGATCCACTGGCTTGTCGATGGC

175  K  E  K  D  A  N  D  T  N  I  H  T  D  L  N  A  I  L  S  K  E  N  T  S  Y  S  I  S  S
     AAGGAGAAAGATGCCAATGACACGAACATTCACACTGATCTCAATGCCATCCTATCAAAAGAAAATACATCTTACAGCATCAGCAGC

204  R  L  R  F  D  A  L  D  W  A  R  S  K  N  V  E  C  R  V  D  L  Y  T  N  E  S  V  P  T
     CGGCTGAGATTCGACGCCCTAGATTGGGCTCGGTCCAAGAACGTTGAGTGCAGAGTAGATCTCTACACTAACGAATCAGTGCCTACA

233  T  S  S  S  T  L  A  V  K  A  E  M  C  G  I  S  K  E  A  K  I  Q  S  M  A  T  A  K  L
     ACAAGCAGTTCAACATTAGCTGTCAAAGCAGAAATGTGCGGTATAAGTAAAGAGGCGAAAATCCAAAGCATGGCAACGGCAAAACTG

262  T  Y  L  I  L  I  C  K  S  I  F  Y  T  I  F  I  S  T  I  A  W  K  T  K  T  S  Y  S  K
     ACATACCTGATCCTCATCTGCAAAAGCATCTTTTATACAATTTTCATCTCAACAATTGCTTGGAAAACTAAGACGTCTTACAGCAAA

291  R  F  D  .
     AGGTTTGACTGAtcgtcgcaaattaatgaactcaaaagatggataagcagcagaac
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FIG. 1. Nucleotide and predicted amino acid (numbered 1–293) sequence of cDNA HF6. Stop codon is indicated as an overdot. Uppercase nucleotides correspond to the predicted coding sequences; lowercase nucleotides designate the 5′ and 3′ untranslated regions. Predicted boundaries of the leader, V, J, and C regions correspond to functional regions of mammalian TCRs. Regions of nucleotide sequence of cDNA HF6 that correspond to the 5′ (WYRQ) and 3′ (YYCA) primers are in boxes.

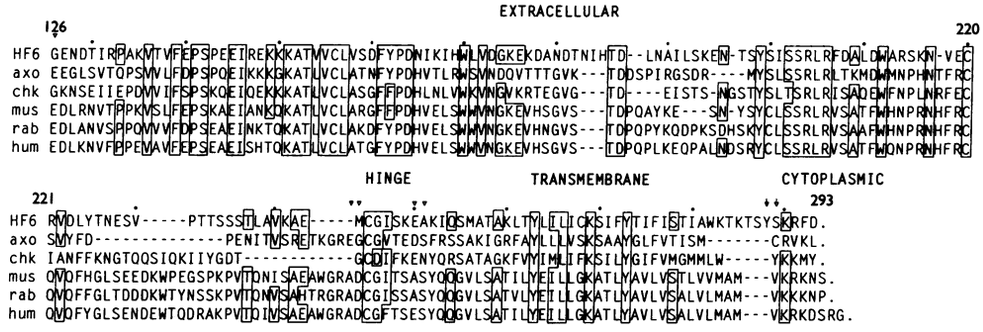

FIG. 2. Alignment of predicted peptide sequence of positions 126–293, corresponding to the C region of the *Heterodontus* HF6 TCR homolog, with the C region sequences of five higher vertebrate TCR β-chain genes from axolotol (axo), chicken (chk), mouse (mus), rabbit (rab), and human (hum). Absolute identity between HF6 and at least three additional TCR β-chain sequences is shown. Assignment of functional regions (boundaries indicated by triangles) is based on mammalian TCR structure. Stop codons are shown by overdots. Gaps (-) were introduced to maximize sequence identity. GenBank identifiers for the specific sequences shown are as follows: axo, AMTCRBC; chk, CHKTCRBCC; mus, MUSTCBXH; rab, RABTCBXB; hum, HUMTCBYY.

nized; however, the short length precludes a more exact homology assignment (see below).

Owing to the considerable sequence relatedness between immunoglobulin and TCR V regions (22, 23), cDNA HF6 is designated as a TCR homolog based on the alignment of the putative C region with the corresponding portions of tetrapod TCR prototypes (Fig. 2). The C region of the shark TCR homolog consists of 168 amino acids as compared with the 154- to 178-amino acid range for the other C regions; this length excludes classification of the cDNA as a light chain (20). In this alignment, 31 positions are fully conserved and an additional 22 are shared by the shark sequence and TCR β-chain genes from at least three of the five other species. A total of 60 HF6 residues are shared with at least two of the five sequences. The highest number of identities is found in the extracellular domain. A functionally significant lysine residue, which interacts with CD3 in mammals (24), is present at position 269 in HF6, as well as in all TCR genes.

Southern blot analyses of CHEF-separated *Heterodontus* DNA are shown in Fig. 3. A high degree of concordance in hybridization with the V_T- and C_T-specific probes, which are unrelated at the nucleotide level, is evident in the *Nru* I and *Mlu* I digests (as well as with *Eag* I and *Sfi* I; data not shown), consistent with multiple V_T–C_T linkage groups. In contrast, *Not* I digestion reveals some nonconcordant hybridization and is consistent with the detection of *Not* I sites in a significant number of V_T^+ λ clones (see below; data not shown). Based on concordant hybridization at ≈50 kb in the *Nru* I digest, at least some V_T and C_T segments are likely within this linkage distance.

A *Heterodontus* genomic λ library (18) was screened with PCR-generated probes complementing the putative V_T segment and the first predicted putative C_T exon of the HF6 cDNA and 191 V_T^+ and 103 C_T^+ plaques were identified; no dual hybridizing (V_T^+/C_T^+) plaques were detected. Twelve V_T^+ genomic clones were restriction mapped and each is unique; eight of these clones contain two V_T segments and 11 hybridize with a J_T probe. Of 12 C_T^+ clones characterized similarly, 10 are unique and each contains a single C_T region. Seven clones are J_T^- and five are J_T^+. Representative maps of four clones are shown in Fig. 4*A*. The large number of C_T-containing clones is reminiscent of the large numbers of immunoglobulin heavy-chain C (25) and light-chain C region-containing (26) clones identified previously in this species, a distinguishing feature of the multicluster form of gene organization. From the λ genomic map analyses, it is evident that (*i*) single V_T-containing clones contain the greatest number of J_T hybridizing elements, up to four if a single V_T maps to a λ arm; (*ii*) of the five J_T^+/C_T^+ λ clones, each lacks the 3′ cytoplasmic exon (determined by hybridization with an exon-specific probe generated from sequence information contained in Fig. 2), consistent with the map placements of C_T near a λ arm; and (*iii*) C_T^+ λ clones containing the 3′ cytoplasmic exon do not contain a detectable J_T segment. Separate screening with J_T-specific probes identified $J_T^+/V_T^-/C_T^-$ clones, consistent with the other screening–mapping results. Nucleotide sequence analyses of representative genomic V_T and J_T segments indicate typical recombination signal sequences (4): 3′-V_THF2VA, CACAGTG–23-bp spacer–TCAAAAACA; 5′-J_THF1JA, GATTTTGTG–12-bp spacer–CAGTGTG. Genomic V_T clone HF2VA is 96% identical (corresponding portion) at the nucleotide level to cDNA HF6. A leader intron is absent from the shark genomic V_T segment, similar to the chicken TCR α-chain locus (8).

Taken together, the library screening, conventional restriction enzyme mapping, intersegmental linkage distance estimates using PCR (data not shown), and the CHEF analyses are consistent with multiple TCR-like clusters, each consisting of multiple V_T and J_T segments and a C_T region. A model of one such cluster is shown (Fig. 4*B*), which does not include the possibility of additional linked V_T and C_T segments, including multiple J_T/C_T segments, the latter analogous to

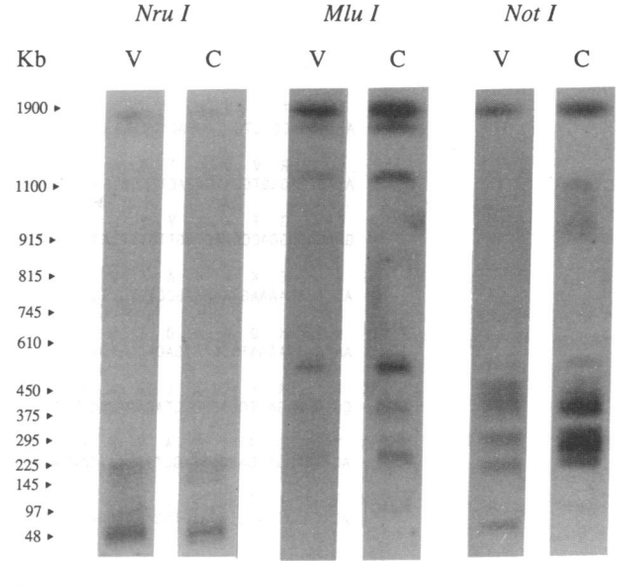

FIG. 3. CHEF analyses of *Heterodontus* agarose-embedded erythrocytes digested to completion with the various restriction endonucleases indicated. Numbers on left represent size standards in kb. Standards are select *Saccharomyces cerevisiae* chromosomes and a λ ladder.

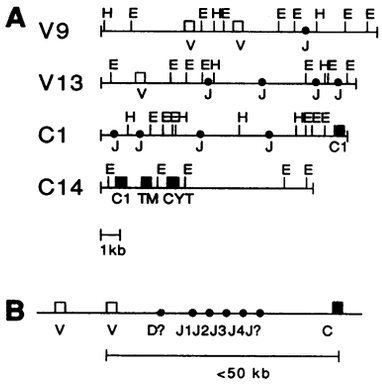

FIG. 4. (*A*) Partial restriction maps of four *Heterodontus* genomic clones selected by hybridization with V_T-specific (V9 and V13) and C_T-specific (C1 and C14) probes. Cleavage sites for *Eco*RI (E) and *Hin*dIII (H) are indicated. Length estimation is by conventional electrophoresis relative to standards and from sizing of products amplified by PCR. □, V_T; ●, J_T; ■, C_T. A probe specific for the extracellular portion of the C region (C1) was generated by PCR based on the sequence relationships shown in Fig. 2; oligodeoxynucleotide probes specific for the transmembrane (TM) and cytoplasmic (CYT) portions of the C regions were used directly. (*B*) Tentative organization of a *Heterodontus* TCR homolog locus based on conventional restriction mapping, intergenic distance estimates determined by PCR, and Southern blot CHEF analyses (see Fig. 3). Map is a composite of results that are entirely consistent with available data for 20 unique λ genomic clones. V, possible D (D?), J segments, and C region exons are indicated; symbols are as in *A*. Placement of D? (presence and number of segments not known) is based on analogy with higher vertebrate systems and is consistent with sequences of recombination signal sequences described in this manuscript. Overall length is shown below and is estimated at <50 kb based on CHEF analyses (Fig. 3). A λ genomic clone has been identified that contains only four J segments (no V or C); additional J segments, not recognized by the J-specific probe, are indicated as (J?).

mammalian systems. Accurate estimations of the number of clusters as well as the number of V_T and C_T elements in a cluster are confounded by limitations in the insert sizes of existing genomic libraries and the possibility that, like immunoglobulin in this species, TCR genes are present at multiple loci on different chromosomes (27, 28). The estimated inter-V–C linkage distance and the presence of multiple V_T and J_T segments associated with a C_T segment differ from immunoglobulin heavy-chain (18, 29) and light-chain (26) gene clusters, which contain single copies of the recombining elements and are ≈18 and ≈2.7 kb in overall length, respectively. The close linkage of V_T and J_T differs from mammalian TCR β-chain loci (30, 31). From the sequence shown in Fig. 1 and by analogy to higher vertebrate TCR β-chain genes, it is reasonable to assume that a D segment(s), possessing 12-nucleotide 5′ and 23-nucleotide 3′ recombination signal sequences, may be present between V_T and J_T (7, 32) in addition to junctional insertion of nucleotide sequence (Fig. 4*B*); alternatively, direct V–J joining may occur.

The question of overall diversity of the TCR homologs has been approached by screening the same cDNA library with PCR-generated C_T- and HF6V_T-specific probes. A large number of hybridizing plaques, C_T^+/V_T^{HF6}, were detected of which four were selected for further characterization. The predicted amino acids of the four V_T segments, of which the sequence of HF15 was truncated, are indicated in Fig. 5. It is evident that a high degree of sequence difference exists among these V_T segments, including HF6. In that these clones were selected solely on the basis of C_T^+, V_T^{HF6} hybridization, it is likely that this family of TCR homologs is extensively diversified. HF6 V region cross-hybridizing clones account for 30% of C_T^+ cDNA clones in this library. V_T region amino acid identity ranges from 24% to 41%, similar to that seen in mammals (33) but in contrast to the very closely related sequences of *Heterodontus* heavy-chain V region genes (12, 18).

DNA cross-hybridization approaches have proven effective in identifying immunoglobulin gene homologs in lower vertebrates (28, 34); however, outside of mammals, only the avian TCR β-chain gene has been identified in this manner (6). The considerable sequence diversity among TCR genes in mammalian species and within the TCR multigene families in a single species (31) suggests that direct screening or PCR amplification with degenerate oligodeoxynucleotide primers may not be generally applicable, although this latter approach was successful with one amphibian species (9). A number of direct screening approaches, including the use of degenerate oligodeoxynucleotide probes as well as PCR amplification using highly degenerate primers, have failed to identify TCR homologs in cartilaginous fishes (refs. 27 and 28; unpublished observations). In addition to the sequences reported here, similar PCR amplifications of genomic DNA from two other cartilaginous fish (*Raja*, skate; *Hydrolagus*, ratfish) and additional vertebrate species (*Gallus*, chicken; *Xenopus*, African clawed frog; *Brachydanio*, zebrafish; *Spheroides*, pufferfish) yield 190- to 200-bp products with significant sequence identity to TCRs. This approach has broader applications than these phylogenetic analyses, as it has the potential for detecting related genes and pseudogenes, including additional TCR families, in which these conserved sequence elements (or variants thereof) are separated by relatively short distances.

The overall complexity and genomic organization of the TCR homologs obtained thus far are characteristic of both the immunoglobulin heavy-chain (18, 29) and light-chain (20, 26) multicluster gene families described in this species and other cartilaginous fishes (28, 35). Parallel evolution of systems that may possess independent physiological roles raises questions about broad aspects of chromosomal organization of genes in this species as well as adaptive advantages and genetic regulation of cluster-type genes.

The presence of TCR-like genes at an early stage of vertebrate evolution provides strong evidence for an integral component of T-cell-type immunity in the phylogenetically primitive vertebrates. The remarkable degree of intergenic variation associated with the respective V regions further suggests functional homology. Ascribing a physiological role for the TCR homologs is less clear as two characteristic properties of T-cell immunity, acute allograft rejections (17) and affinity maturation (T-cell-dependent B-cell maturation) (36, 37), are not associated with immune responses in this species; however, T-cell help in the initial phase of B-cell clonal expansion may be present. Although convincing evidence has been presented for a somatic mutation or some type of gene conversion in this species (38), it appears that the mechanism(s) for selecting such mutants is not operative, at

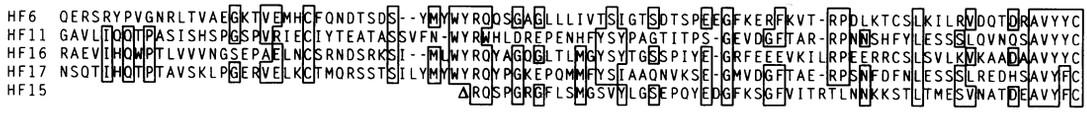

FIG. 5. Alignment of *Heterodontus* TCR cDNA V region predicted amino acid sequences [through Y(Y/F)C]. Amino acid identities among three or more sequences are boxed. HF15 is experimentally deleted Δ—i.e., a 5′-truncated clone.

least within the context of the analyses that have been carried out to date. We have ascribed this to an absence of germinal centers (38), which are the selection site of somatic mutation in higher vertebrates (39, 40). Thus, it seems that the cellular selection mechanisms for somatic mutations were an adaptation later than the mechanism(s) that actually generates diversity. The physiological roles of both the immunoglobulin and TCR-like systems in the primitive vertebrates remain uncertain, but their presence at such an early stage of vertebrate phylogeny strengthens the hypothesis that the structurally homologous gene may be responsible for other forms of historecognition in the more phylogenetically distant jawless vertebrates, protochordates, and invertebrates. The results of these studies as well as other data being accumulated in ongoing parallel investigations of other species are providing information as to the nature and extent of variation in short, shared sequence regions that are amenable to the type of amplification reactions described here, affording a possible means for detecting TCR-like homologs in more distant forms.

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