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Immunology

Evolutionary relationship between the T3 chains of the T-cell receptor complex and the immunoglobulin supergene family

(T-lymphocyte-specific membrane proteins/murine T3-ε chains/secondary structure predictions/immunoglobulin domains)

DANIEL P. GOLD*, HANS CLEVERS*, BALBINO ALARCON*, SABRINA DUNLAP*, JIRI NOVOTNY†, ALAN F. WILLIAMS‡, AND COX TERHORST*

*Laboratory of Molecular Immunology, Dana–Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, MA 02115; †The Laboratory of Cellular and Molecular Research, The Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114; and ‡Medical Research Council, Cellular Immunology Research Unit, Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, United Kingdom

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ABSTRACT **Antigen receptors on the surface of the thymus-derived (T) lymphocytes are associated with small integral membrane proteins called the T3 (CD3) γ, δ, ε, and ζ chains. After interaction of the T-cell receptor with antigen, the T3 proteins are believed to transfer an activation signal to the intracellular compartment. In previous studies, the human γ, ε, and δ chains have been cloned along with the mouse δ chain, but a relationship between these sequences and known molecular families has not been established. We now report the molecular cloning and characterization of the murine T3-ε protein and a sequence and structural analysis of the relationships between all the T3 chains and the immunoglobulin superfamily. It is established that the T3 chains are immunoglobulin-related and a particular relationship to the neural cell adhesion molecule (N-CAM) is noted. This sequence relationship adds interest to previous findings that the T3 chains are genetically linked to N-CAM and Thy-1 antigen on band q23 of human chromosome 11.**

The classical T-cell receptors (TCR) for antigen (α/β heterodimer) are intimately associated with the T3 (CD3) proteins (T3-γ, -δ, -ε, and -ζ) (1–4). Recently, several groups have described a subpopulation of thymus-derived lymphocytes that express TCR-γ and -δ chains associated with T3 proteins (5, 6). In addition, on the surface of a human T lymphocyte clone with natural killer-like activity, we have found a TCR-γ homodimer that is also coexpressed with the T3 polypeptide chains (7).

Information about the protein and gene structure of the TCR–T3 polypeptide chains will aid in our understanding of the function, assembly, and tissue-specific expression of the complex. Isolation and characterization of the human T3-γ, -δ, and -ε cDNAs has been reported (8–10). Here we analyze the sequence of the murine T3-ε chain and assess the relationship between the T3 family and the immunoglobulin supergene family.§

METHODS

Isolation of a Murine T3-ε cDNA Clone. Clone DL1 was isolated from a mouse T-cell cDNA library constructed in phage λgt10 (11) using the insert from pDJ1 as probe (10). Twelve positive bacteriophages were plaque purified and their insert sizes were determined by standard techniques (12).

DNA Sequence Determination. Restriction fragments of the DL1 clone were subcloned in the bacteriophage M13 and were used for sequencing with the dideoxy chain-termination method (13).

In Vitro **Translation of Mouse T3-ε RNA.** *In vitro* transcription by SP6 RNA polymerase was carried out in a 25-μl reaction mixture using 9.5 μg of *Pvu* II-linearized pDL1 according to the manufacturer's specifications (Promega Biotec) in the presence of 50 μM GTP and 500 μM diguanosine triphosphate (P-L Biochemicals). The RNA was then phenol extracted and precipitated in ethanol for translation in wheat germ extract using 20 μCi of [^{35}S]cysteine (1 Ci = 37 GBq) according to the manufacturer's specifications (Amersham). ^{125}I-labeled T3 proteins were precipitated from DO-11.10 cells as described (3). NaDodSO$_4$/PAGE was by standard methods (3).

Protein Sequence Comparisons. For comparisons between sequences, segments thought to be related to an immunoglobulin domain were defined by taking 20 amino acids before and after cysteine residues that were considered equivalent to the cysteine residues of the conserved disulfide bond in immunoglobulin domains. In T3-δ, there are only 15 residues prior to the NH_2-terminal cysteine and thus leader sequence residues were included for the statistical analyses. This was also done for any other sequence with <20 amino acids prior to the first conserved cysteine. The statistical significance of similarities between sequences was assessed by using the ALIGN program (14) with scores based on the mutation data matrix and a bias and gap penalty of 6 and 100 or 150 random runs.

References to sequences are cited or given as the code numbers in the data base from the Protein Identification Resource.¶

RESULTS AND DISCUSSION

The Mouse T3-ε Sequence. Complementary DNA encoding the mouse T3-ε chain was identified by screening a λgt10 cDNA library from mouse T cells with a ^{32}P-labeled probe from human T3-ε cDNA (10). One clone called pDL1 contained an insert of 1.5 kilobases, which is about the size of mouse T3-ε mRNA (10), and this gave a sequence of 1438 base pairs as shown in Fig. 1. A consensus poly(A) addition sequence of AATAAA began at nucleotide 1419.

The translated sequences from the mouse and human T3-ε chain are shown in Fig. 2 with a putative transmembrane sequence dividing the chains into postulated extracellular (NH_2-terminal) and the cytoplasmic (COOH-terminal) seg-

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Abbreviations: TCR, T-cell receptor; N-CAM, neural cell adhesion molecule; V and C, variable and constant regions of immunoglobulin; β_2m, β_2-microglobulin; MHC, major histocompatibility complex.

§The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02990).

¶Protein Identification Resource (1985) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 8.0.

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cgcgtctggtgccttcttcagaaatgaagtaatgagctggctgcgtccgccatcttggtagag 63
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AGC CTC CTA GCT GTT GGC ACT TGC CAG GAC GAT GCC GAG AAC ATT GAA 162
TAC AAA GTC TCC ATC TCA GGA ACC AGT GTA GAG TTG ACG TGC CCT CTA 210
GAC AGT GAC GAG AAC TTA AAA TGG GAA AAA AAT GGC CAA GAG CTG CCT 258
CAG AAG CAT GAT AAG CAC CTG GTG CTC CAG GAT TTC TCG GAA GTC GAG 306
GAC AGT GGC TAC TAC GTC TGC TAC ACA CCA GCC TCA AAT AAA AAC ACG 354
TAC TTG TAC CTG AAA GCT CGA GTG TGT GAG TAC TGT GTG GAG GTG GAC 402
CTG ACA GCA GTA GCC ATA ATC ATC ATT GTT GAC ATC TGT ATC ACT CTG 450
GGC TTG CTG ATG GTC ATT TAT TAC TGG AGC AAG AAT AGG AAG GCC AAG 498
GCC AAG CCT GTG ACC CGA GGA ACC GGT GCT GGT AGC AGG CCC AGA GGG 546
CAA AAC AAG GAG CGG CCA CCA CCT GTT CCC AAC CCA GAC TAT GAG CCC 594
ATC CGC AAA GGC CAG CGG GAC CTG TAT TCT GGC CTG AAT CAG AGA GCA 642
GTC TGAcagataggagagacatcgccttctgtggacccagatccagccctccgagcaccctg 704
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tctctgctaatgcaaaaggcaataaagtgtattggctgga_n 1438
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FIG. 1. Murine T3-ε nucleotide sequence. The nucleotide sequence of pDL1 was determined by the dideoxy chain-termination method of Sanger *et al.* (13) using bacteriophage M13mp18 or M13mp19. The putative AATAAA poly(A) addition site cDNA is underlined.

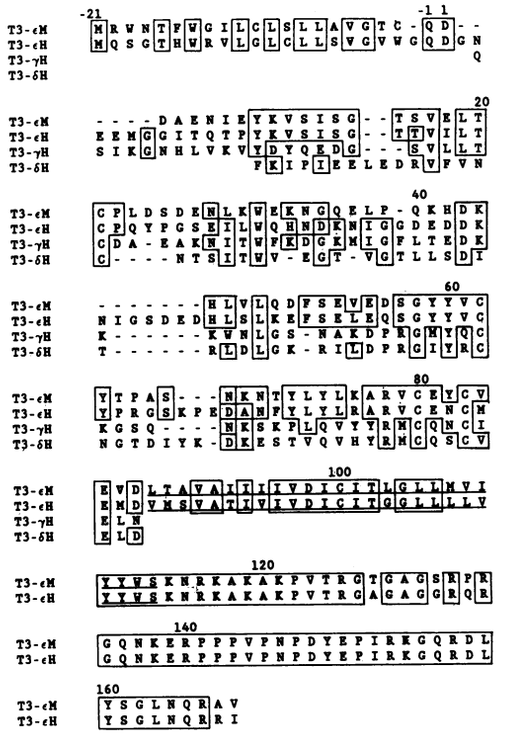

FIG. 2. Alignment of T3-ε sequences with other T3 sequences. Comparison of mouse (M) and human (H) T3-ε sequences (8–10). Boxes show positions of identity between T3-εM and T3-εH and positions where a residue in T3-γH or T3-δH matches either of the T3-ε sequences. The putative transmembrane sequences in T3-εM and T3-εH are underlined. Amino acids are identified by the single-letter code.

ments. The murine chain is predicted to contain 168 amino acids (M_r, 19,140) compared with 185 for the human with the difference in length being found in the extracellular part, which contains 87 and 104 residues in each case (10). The extracellular part shows 47% identity compared with 65% in the transmembrane sequence and 91% in the cytoplasmic domain. The high level of identity in the cytoplasmic domain suggests important interactions with other cytoplasmic molecules. The preservation of the unusual feature of an aspartic acid in the transmembrane sequence is in accord with the notion that this residue, along with the charged residues in the transmembrane segments of other chains of the TCR–T3 complex, plays a role in the stability of the complex.

Expression *in Vitro* of Mouse T3-ε cDNA. When the TCR complex of mouse T cells is examined by surface labeling with ^{125}I and immunoprecipitation, five associated bands are seen, as shown in Fig. 3 (lane B) (3, 4). To see which of these corresponded to the mouse T3-ε chain, the T3-ε cDNA was transcribed and translated *in vitro* using the SP6 system. The radiolabeled translation product ran on $NaDodSO_4$/polyacrylamide gel with an apparent M_r of 20,600, including the leader peptide, which leads to an estimate of 18,200 for the processed polypeptide—in reasonable agreement with the predicted M_r of 19,140. It seems likely that this product corresponds to band 2 of the surface-labeled chains since the T3-ε chain lacks N-linked glycosylation sites and band 2 is the only surface-labeled chain that does not shift in apparent M_r on digestion with endoglycosidase F (3). The discrepancy of ≈5000 in apparent M_r between the *in vitro* translation product and band 2 might be due to O-linked oligosaccharides because band 2 can be labeled with mannose (3) and undergoes a shift in M_r after treatment with trifluoromethanesulfonic acid (B.A., unpublished data). As with band 2 (3), the *in vitro* translation product of cDNA clone pDL1 has a considerably lower mobility under reducing conditions than under nonreducing conditions (Fig. 3 *Lower*). Unlike bands 4 or 5 (the so-called T3-ζ), the mouse T3-ε chain does not form disulfide-linked homodimers (Fig. 3 *Lower*).

Sequence Similarities Between the T3 Chains. Previous studies noted a strong relationship between T3-δ and -γ chains (9) but similarities of either of these chains to the T3-ε chains are less striking although patches of similarities close to the transmembrane sequences (residues 78–86 in T3-εM) were identified (10). Alignments of extracellular parts of T3-δ and -γ with T3-ε (Fig. 2) show identities around the cysteine residues including the strong similarities adjacent to the transmembrane sequence in all chains.

The significance of matches between pairs of sequences was scored using the ALIGN program, which determines the best sequence alignments and scores them in terms of standard deviation (SD) units. Details of the scores are given in the legend to Fig. 4. Dayhoff *et al.* (14) regard scores of >3 as indicating the possibility that sequences may be related in evolution. If comparisons are made with a family of sequences, repeated significant scores with different sequences will indicate that matches are being made with a pattern of residues that is conserved within the family of sequences. Fig. 4*A* shows that high ALIGN scores are obtained when extracellular T3 sequences up to the transmembrane sequence are compared and these argue strongly for an evolutionary relationship.

Comparisons with Immunoglobulin Family Sequences. In all T3 chains, there are cysteine residues that might form disulfide bonds like the conserved disulfide bond in sequences of the immunoglobulins (Cys-21 and -62 in mouse T3-ε sequence). A significant relationship between human

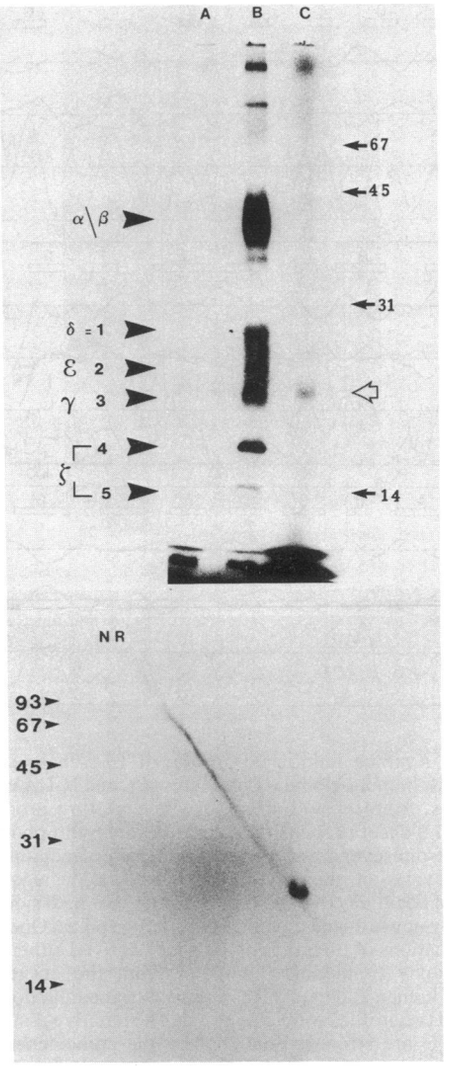

FIG. 3. (*Upper*) Comparison of the *in vitro* translation product of mouse T3-ε from SP6-generated RNA and the murine cell-surface TCR–T3 complex. All surface radioiodinated TCR–T3 proteins were analyzed by $NaDodSO_4$/PAGE (lane B). Lane A, analysis after precipitation with a nonimmune serum. The SP6 translation product labeled with [^{35}S]methionine is shown in lane C. (*Lower*) Two-dimensional $NaDodSO_4$/PAGE analysis of *in vitro* synthesized mouse T3-ε. Proteins were analyzed under nonreducing conditions (NR), followed by analysis under reducing conditions (indicated by the molecular weight markers $\times 10^{-3}$).

A

	—T3:TO TRANSMEMBRANE— εM	εH	γH	δH	—T3-AS FOR IG COMPARISONS— εM	εH	γH	δH
T3-εM	---	16.8	5.9	6.1	---	13.3	4.3	3.4
T3-εH	16.8	---	7.1	6.0	13.3	---	4.5	3.8
T3-γH	5.9	7.1	---	12.0	4.3	4.5	---	9.5
T3-δH	6.1	6.0	12.0	---	3.4	3.8	9.5	---

B

	——IgV—— Hκ	Hλ	HV_H	————TCR V———— Hβ	Mβ	Hα	Mα	Mγ	————V-RELATED SEQUENCES——— HCD4	RCD8 I	RCD8 II	ROX-2 I	RThy1	PIgR I	PIgR III
T3-εM	1.6	1.6	1.5	3.5	4.4	1.6	2.4	1.8	3.3	2.6	1.5	3.3	3.4	1.7	2.3
T3-εH	2.3	2.2	1.4	4.3	6.0	3.2	3.1	2.7	4.7	2.1	3.8	3.1	1.9	2.8	0.7
T3-γH	2.4	1.1	1.4	2.4	2.9	2.1	2.9	1.4	4.9	1.9	1.0	-.8	1.5	1.6	2.0
T3-δH	3.7	0.3	0.7	2.0	1.3	1.9	1.0	1.9	2.6	1.3	-.3	0.4	0.2	1.2	0.2
THY-1	4.2	7.0	4.0	3.8	2.9			1.1	3.2	4.6	3.1	5.0		5.9	6.7
T4	7.4	4.0	6.3	5.1	7.0			4.9		5.4	7.4	1.8	3.2	5.9	5.9

C

	——MHC ANTIGENS—— β_2-M	I	II	II	————Ig-C——— λ	κ	C_H1	C_H2	C_H3	——TCR-C—— Cα	Cβ	Cγ	———N-CAM——— II	III	IV
T3-εM	1.2	4.4	1.7	1.9	0.6	1.1	2.0	-.2	1.6	2.0	1.2	-.2	4.2	5.8	2.5
T3-εH	2.3	2.3	3.6	3.8	2.7	3.7	3.8	0.3	2.0	2.3	2.7	1.8	6.0	7.6	4.5
T3-γH	2.1	2.8	4.0	4.6	1.3	2.2	3.0	1.6	1.1	2.0	0.9	0.8	3.6	3.7	5.4
T3-δH	1.9	1.7	2.0	3.4	2.5	2.7	2.5	-.8	2.3	1.3	2.8	1.4	2.9	3.2	2.3
β_2M	---	8.9	11.2	12.6	5.7	5.7	4.4	2.9	5.5				5.1	2.7	3.7
MHC I	8.9	---	10.1	11.1	7.4	8.2	7.1	3.9	8.7				5.7	2.9	3.6

FIG. 4. ALIGN scores for comparisons between T3 and immunoglobulin supergene family sequences. Sequences were chosen for ALIGN program comparisons as described except for the first set of T3 comparisons, where sequences were extended to the position where the transmembrane sequence begins. Sequences with (references) or [NBRF code names] are as follows: Hκ, immunoglobulin V_κ [K1HURY]; Hλ, immunoglobulin V_λ [L1MS4E]; HV_H, immunoglobulin V heavy chain [G1HUNM]; Hβ, human TCR Vβ [RWHUVY]; Mβ, mouse TCR Vβ TB12 (15); Hα, human TCR Vα [RWHUAV]; Mα, mouse TCR Vα [RWMSAV] HCD4, human HCD4 domain I (16); RCD8I and II, rat CD8 32-kDa and 37-kDa chain (17); R OX I, rat OX 2 domain I (18); R Thy-1, rat Thy-1 [TDRT]; P immunoglobulin R I and III, rabbit polyimmunoglobulin receptor domains I and III [QRGBG]; β_2m, human β_2m [MGHUB2]; MHC I, human MHC class I α3 domain [HLHU12]; MHC II α and β, MHC II DR α and β chain [HLHUDA] and [HLHU3D]; immunoglobulin C domains, light chain λ [L2HU], light chain κ [K3HU], immunoglobulin γ1 C_H1, C_H2, and C_H3 all [GHHU]; TCR-C domains, α[RWHUAC], β[RWHUCY], γ[RWMSCI]; N-CAM domains II-IV (19).

T3-ε chain and some of the immunoglobulin-related domains was seen in terms of ALIGN scores (Fig. 4). The scores for the comparisons can be assessed by relating them to those for other cell-surface antigens that are accepted as being variable region (V)-related (Thy-1 and T4) (16, 20) or constant region (C)-related [β_2-microglobulin (β_2m) and class I major histocompatibility complex (MHC)] (15), which are also given in Fig. 4.

Among the V-related set, the best match of all was between human T3-ε and the mouse TCR-β chain TB12 (15); the sequence alignment for this is shown in Fig. 5*a*. Between the TCR sequence and human T3-ε, there are 29 identities of 89 aligned residues with 6 sequence breaks. This is a high level of identity for sequences within the immunoglobulin superfamily.

From the ALIGN scores of the C-related set in Fig. 4*C*, the matches with neural cell adhesion molecule (N-CAM) are impressive both in the level of the scores and the fact that significant scores are obtained with three sets of N-CAM sequences (domain I of N-CAM also gave significant scores but was not used since sufficient sequence was not available at the NH_2-terminal end) (19). Alignments of the T3-ε sequences with N-CAM domains II and III are shown in Fig. 5*b* and it can be seen that a large gap in the middle of the sequence is not necessary, particularly with human T3-ε and the N-CAM sequences. The N-CAM pattern on the NH_2-terminal side of the second cysteine is like that seen in V- and V-related sequences, yet N-CAM would not be likely to have a V-domain folding pattern (19).

The immunoglobulin and TCR C-domain sequences gave poor scores with T3, but the matches of T3 with MHC class I and II antigens were more extensive. Some impressive patches of sequence identity were seen in this group, as shown in Fig. 5*c*, where alignments in the region of β-strand C are shown for IgG C_{H3}, β_2m, and class II MHC antigen domains.

All immunoglobulin domains share the same folding scheme and the immunoglobulin fold can be described as a consecutive sequence of seven β-strands, A–G, arranged in two antiparallel β-sheets packed face to face: A-B-D-E and C-F-G (21–23). Each of the β-strands has characteristic amino acid residues at positions where the side chains point inside the sheet. All these structural features translate into characteristic patterns of hydrophobicity, α-helix, β-sheet and reverse-turn propensities, and electric charges. These patterns, particularly when computed and averaged from several amino acid sequences, have been used successfully in

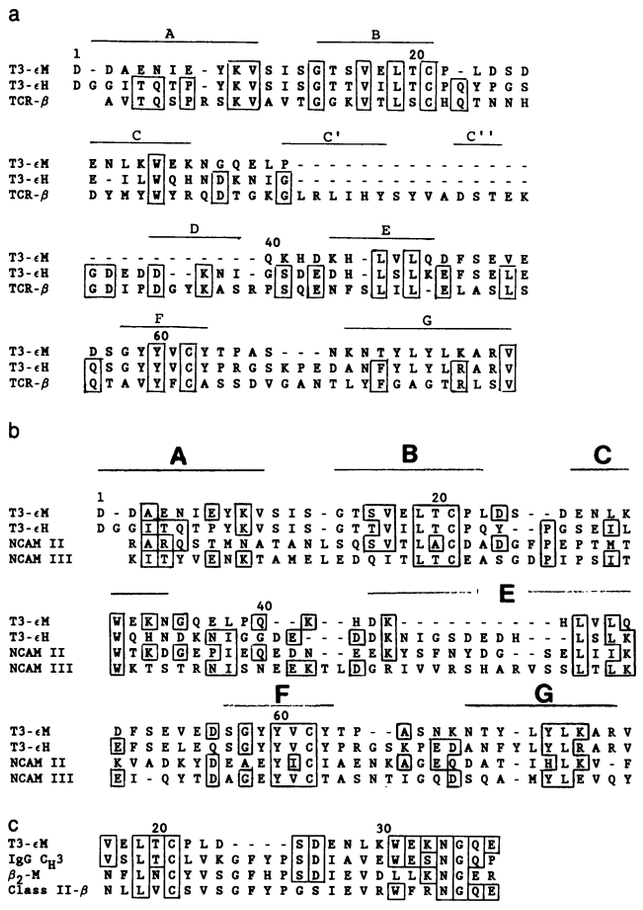

FIG. 5. (*a*) Comparison of T3-εM and T3-εH with the TCR TB12 β-chain sequence (15). Positions where the TB12 sequence matches either of the T3 sequences are boxed. Positions for postulated β-strands determined by comparison of the TCR sequence with immunoglobulin V domains of known tertiary structure (21) are shown above the T3-εM sequence and are labeled A, B, C, C′, C″, D, E, F, and G (20). (*b*) T3-ε sequences aligned with N-CAM sequences. Domains II and III of N-CAM as in ref. 19 are aligned with mouse (M) and human (H) T3-ε sequences. Residues are boxed where any N-CAM sequence matches a T3-ε sequence. (*c*) Comparisons of mouse T3-ε sequences with C domains and MHC antigen sequences. Comparisons are made in the region of the cysteine of β-strand B and tryptophan of β-strand C in the immunoglobulin fold (see Fig. 2*A*). Residues are boxed where the T3-εM sequence matches any of the others. NBRF data base codes are for the sequences given in the legend to Fig. 4. Amino acids are identified by the single-letter code.

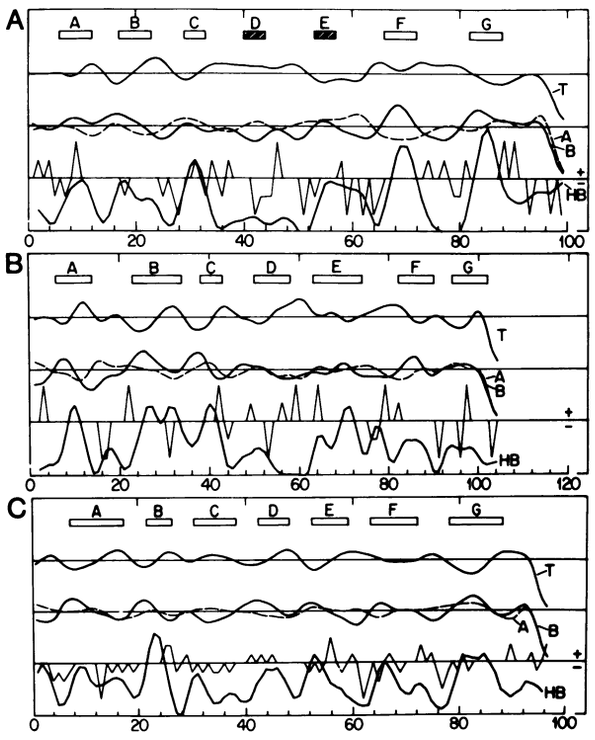

FIG. 6. Average amino acid profiles of T3-ε-chain extracellular domains (*A*), immunoglobulin C domains (*B*), and N-CAM molecules (*C*). Curves, from top to bottom, are reverse turn propensity (T), α-helix and β-sheet propensity (A and B), and hydrophobicity (HB) computed from several aligned sequences, averaged, and smoothed by three cycles of the 7-point moving window algorithm (24). Positions of positively (+) and negatively (−) charged side chains are indicated by upward- and downward-pointing spikes. Open bars A–G indicate locations of β-strands or α-helices derived either from x-ray crystallography (immunoglobulins) or from the curves. (*A*) Sequences of human and mouse T3-ε-chain extracellular domains were aligned and the profiles were computed. The putative β-strands A, B, C, F, and G are strongly predicted by the coincidences of sharp hydrophobicity maxima, β-propensity peaks, and minima in the turn profiles. The identity of the elements D and E is more ambiguous (either α-helices or β-strands). (*B*) Sequences of mouse and human immunoglobulin light chain C regions were aligned and the profiles were computed. (*C*) Sequences of the four domains of the N-CAM molecule, aligned as by Hemperly *et al.* (19), were used to compute average profiles. The profiles are analogous to those of the immunoglobulin domains shown in *B*.

secondary structure predictions of members of the immunoglobulin supergene family (23, 24). The secondary structure predictions of T3-ε (Fig. 6*A*) indicate a presence of five or perhaps six β-strands. These are in approximately the same position as in C region domains of immunoglobulin light chains (Fig. 6*B*) and as in N-CAM domains (Fig. 6*C*). When all four N-CAM domains were aligned, their average profile (Fig. 6*C*) is a clear example of a 7-stranded β-sheet bilayer. Given the sequence homology of T3-ε with the N-CAM domains, we conclude that T3-ε also belongs to this fold (Fig. 6*A*). From this analysis, T3-ε could make a fold like a C domain with sheets A, B, E and G, F, C plus a short connecting piece across the top of the two sheets (see also Fig. 5*a*). The missing strand D, which is absent in T3, is at the edge of the β-sheet and thus need not be present to maintain the basic fold.

The fact that T3 and N-CAM are related is particularly interesting since it establishes a relationship between molecules that are part of the T-lymphocyte antigen receptor complex and a sequence that, like Thy-1, is involved in neural cell interactions. Of particular note is the fact that genes for the T3-γ, -δ, and -ε chains are found together with N-CAM and Thy-1 antigen on band q23 of human chromosome 11 and that this linkage also holds in the mouse (25–27). The sequence similarities along with the genetic linkage data suggest the q23 region of chromosome 11 as a genetic region of outstanding interest with regard to evolution of the immunoglobulin superfamily.

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