Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2550–2554, March 1991
Immunology

Primary structure and ontogeny of an avian CD3 transcript

(chicken/phylogeny/subtracted probes/T-cell receptor/thymus)

ALAIN BERNOT* AND CHARLES AUFFRAY

Institut d'Embryologie Cellulaire et Moléculaire du Centre National de la Recherche Scientifique et du Collège de France, 49bis avenue de la Belle Gabrielle, 94736 Nogent sur Marne Cedex, France

Communicated by Nicole M. Le Douarin, December 10, 1990 (received for review October 10, 1990)

ABSTRACT **The structure of a chicken CD3 chain has been determined by isolating a cDNA clone (T11.15) that encodes a 175-amino-acid-long protein, including the NH_2-terminal signal peptide. In Northern blot experiments, the earliest expression of the T11.15 transcript was detected in the thymus at embryonic day 10 {i.e., 1 day after cytoplasmic expression of a CD3 epitope recognized by a specific monoclonal antibody [CT3; Chen, C. L. H., Ager, L. L., Gartland, G. L. & Cooper, M. D. (1986) *J. Exp. Med.* 164, 375–380], but 2 days before the appearance of clonotypic components of the T-cell antigen receptor}. Sequence similarity of this chicken protein sequence compared with that of the known mammalian CD3 γ and δ polypeptides was 36–39% and 39–40%, respectively. Amino acid sequence alignments between avian and mammalian CD3 revealed maximum conservation in the transmembrane and cytoplasmic domains as well as in the regions flanking the cysteine residues in the extracellular domain, underlining their functional importance. The difficulty of unambiguously assigning this chain to a single mammalian CD3 subunit on the basis of sequence comparison raises the possibility that this polypeptide represents a derivative from an ancestral form of the γ and δ chains. It is thus possible that a single chain may play the role of both CD3 γ and δ subunits in the chicken CD3 complex or, alternatively, that gene duplications occurred independently in the avian and mammalian lineages.**

Antigen recognition and response by T lymphocytes are mediated by a group of integral membrane proteins consisting of a clonotypic element referred to as the T-cell antigen receptor (TCR), which is noncovalently linked to several invariant chains of the CD3 complex. The TCR is made up of two 40- to 45-kDa variable glycoproteins (either $\alpha\beta$ or $\gamma\delta$) and is the antigen recognition unit of the complex: each $\alpha\beta$ combination recognizes specifically an antigenic peptide presented by a major histocompatibility complex molecule (1). This interaction is helped by CD4 or CD8, which serves as coreceptor for major histocompatibility complex class II or class I products, respectively (2). The invariant chains consist of at least five subunits: γ (21–25 kDa), δ (20–28 kDa) (only γ and δ are glycosylated), ε (20–25 kDa), and ζ (16 kDa), which exists as disulfide-linked homodimers or heterodimers with the η chain (21 kDa) (3, 4). The γ, δ, and ε chains are collectively referred to as the CD3 complex. These invariant components are involved in signal transduction, which ultimately leads to T-lymphocyte activation after the initial recognition steps (5, 6). Two activation pathways are independently regulated: The first involves the $p56^{lck}$ tyrosine kinase, which is physically associated with the cytoplasmic domain of CD4 or CD8 and catalyzes the phosphorylation of the ζ subunit as well as other, as yet unknown, cytoplasmic proteins. The second pathway involves inositol phospholipid hydrolysis into inositol trisphosphate (which induces an elevation in intracellular free calcium concentration) and diacylglycerol (an activator of protein kinase C, which in turn participates in the phosphorylation of the CD3 γ-chain cytoplasmic region).

The genes encoding each of these subunits have been characterized in humans and mice (reviewed in refs. 1 and 2; see also refs. 7–9). The TCR chains are encoded by immunoglobulin-like gene segments that rearrange during intrathymic T-cell differentiation, whereas the genes encoding invariant components of the CD3 complex do not rearrange. The primary structure of these polypeptides, derived from the nucleotide sequence of the respective cDNAs, has shown that all TCR/CD3 components, except ζ and η, belong to the immunoglobulin superfamily (10).

Among nonmammalian vertebrate species, the best characterized TCR is that from the chicken (*Gallus gallus*). Several monoclonal antibodies have been used to characterize immunochemically surface antigens present on avian T cells that resemble the mammalian TCR/CD3 complex; two types of clonotypic heterodimers, $\gamma\delta$ (TCR1; ref. 11) and $\alpha\beta$ (TCR2; ref. 12), each composed of two disulfide-linked glycoproteins of 50 kDa and 40 kDa, are noncovalently associated with a minimum of three integral membrane proteins (13). Two of them are glycoproteins of 20 kDa and 19 kDa (both having a 16-kDa protein backbone), and one is a 17-kDa nonglycosylated protein. In this study we report the isolation and characterization† of a chicken cDNA that encodes one of these CD3-like proteins. In addition, we describe the appearance of this CD3 transcript during thymocyte development.

MATERIALS AND METHODS

Cell Analysis. The two chicken lymphoblastoid cell lines (LCLs) RP9 and MSB1 were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 2% chicken serum (BRL) at 37°C in a humidified atmosphere containing 5% CO_2. After two washes in phosphate-buffered saline (PBS), 5×10^5 cells were incubated with 100 μl of appropriate monoclonal antibody dilutions in PBS supplemented with 0.1% bovine serum albumin (BSA) for 30 min at 4°C. After three washes in PBS/0.1% BSA, cells were incubated with fluorescein-coupled goat anti-mouse immunoglobulin for 30 min at 4°C, followed by cytofluorometric analysis with a FACS 440 (Becton Dickinson). The monoclonal antibodies CT3 and TCR2 (12, 13) were gifts of Max Cooper (University of Alabama at Birmingham).

RNA Purification. Cells were collected by centrifugation, washed in PBS, and frozen in liquid nitrogen. Thymuses, bursae, spleens, liver, and brain from White Leghorn chickens were preserved in liquid nitrogen. Total RNA was isolated by

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LCL, lymphoblastoid cell line; TCR, T-cell antigen receptor; BSA, bovine serum albumin.

*To whom reprint requests should be addressed.

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M59925).

homogenization in 4 M guanidium thiocyanate, followed by ultracentrifugation through a 5.7 M CsCl cushion (14). Poly(A)$^+$ mRNA was purified by repeated affinity chromatography with an oligo(dT)-cellulose column (15). mRNA from the human T-LCL CEM was a gift from Dominique Piatier-Tonneau (Institut d'Embryologie, Nogent, Marne).

Subtracted Probes. Two hybridization probes were used. The first (T-B) was prepared by subtraction of the MSB1 cDNA with mRNA from RP9, and the control probe (B-B) was prepared by subtraction of the RP9 cDNA with its template. Five micrograms of poly(A)$^+$ RNA (from the MSB1 cell line for the T-B probe or from the RP9 cell line for the B-B probe) was heat denatured and incubated in 50 mM Tris·HCl, pH 8.3/40 mM KCl/8 mM $MgCl_2$/BSA at 100 μg/ml/14 mM 2-mercaptoethanol/4 mM sodium pyrophosphate/1 mM dGTP/1 mM dTTP/160 μM dATP/160 μM dGTP/100 μCi (1 Ci = 37 GBq) of [α-^{32}P]dATP/100 μCi of [α-^{32}P]dCTP (each at 3000 Ci/mmol), supplemented with avian myeloblastosis virus reverse transcriptase (25 units/μg of mRNA) at 42°C for 75 min. After alkaline hydrolysis of the RNA, the mixture was neutralized, phenol extracted, and purified by filtration on a Sephadex G-50 column (16). The single-stranded cDNA was precipitated, heat denatured, and hybridized to 30 μg of poly(A)$^+$ RNA from the RP9 cell line in 10 μl of 120 mM sodium phosphate, pH 6.8/800 mM NaCl/10 mM EDTA/poly(U) (10 μg/ml) at 68°C to a R_0t of 1500 mol·sec·liter^{-1} (17). The material was fractionated by chromatography through a hydroxylapatite column (Bio-Rad; DNA grade, thermostated at 60°C); the single-stranded cDNA was selectively recovered in 120 mM sodium phosphate, pH 6.8/0.05% SDS. Seven to 10% of the input cDNA was recovered in the single-stranded fraction.

Differential Screening. An MSB1 cDNA library in the phage λgt10 vector (a gift from Rima Zoorob, Institut d'Embryologie, Nogent, Marne) was screened with the subtracted probes on duplicate filters (Schleicher & Schuell), in 50% formamide/6× SSC/5× Denhardt's solution/poly(A) at 0.1 mg/ml/poly(C) at 0.1 mg/ml/denatured salmon sperm DNA at 0.1 mg/ml/0.1% SDS (1× Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% BSA; 1× SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7). The first set of replicas was hybridized with the T-B probe; the second set was hybridized with the B-B control. A total of 5×10^5 plaques were screened. Final washes were performed at 65°C in 0.1× SSC/0.1% SDS. Filters were autoradiographed by using Kodak X-Omat AR5 films and intensifying screens for up to 1 month. Putative T-specific clones were defined as clones recognized by the T-B probe but not recognized by the B-B probe. Phage inserts were amplified by the polymerase chain reaction with two 24-mer oligonucleotides corresponding to the sequences flanking the *Eco*RI insertion site of λgt10 (18). After fractionation by agarose electrophoresis, the expression pattern of each amplified insert was assessed by Northern blot analysis.

Northern Blot Analysis. Five micrograms of each sample of mRNA or total RNA was denatured with glyoxal, size fractionated by agarose electrophoresis, and transferred to a nylon membrane (NEN; GeneScreen). Blots were prehybridized in 50% formamide/50 mM sodium phosphate, pH 6.5/5× SSC/1× Denhardt's solution/denatured salmon sperm DNA at 0.1 mg/ml. Hybridizations were performed in the same solution containing 10^5 cpm of random-primed DNA probe per ml at 42°C for 16 hr, followed by washing in 0.2× SSC/0.1% SDS at 65°C (19). Blots were subsequently rehybridized to an actin probe, confirming that comparable amounts of RNA had been loaded in all lanes.

cDNA Cloning and Analysis. Because of the relatively short insert size in the λgt10 library, we constructed a new MSB1 cDNA library in the pCDM8 vector (20) according to the manufacturer's protocol (InVitrogen, San Diego). This library was electroporated (21) in MC1061/P3 cells. The polymerase chain reaction-amplified fragment, which gave a T-cell specific signal in Northern blot (T11), was used as a ^{32}P-labeled probe to screen 300,000 clones of the unamplified library according to standard techniques (19). Three overlapping clones were therefore isolated and sequenced on both strands by the dideoxynucleotide chain-termination method (22). Reactions were carried out with Sequenase (United States Biochemical) according to the manufacturer's recommendations. Synthetic oligonucleotide primers were generated as needed on a nucleotide synthesizer (Applied Biosystems model 381A). Searches for protein homologies with the National Biomedical Research Foundation protein data base (release 25) were carried out using the program KANEHISA (23). Protein sequences were aligned using the CLUSTAL algorithm (24). Corresponding nucleotide sequence alignments were derived from the protein alignments, and dendrograms were calculated based on the Fitch–Margoliash and least-squares methods (25). Computer analyses were performed using the Base Informatique sur les Séquences d'Acides Nucléiques pour les Chercheurs Européens facility (CITI2, Paris).

RESULTS

Isolation of T-Cell-Specific Sequences. Flow cytometric analysis of chicken LCLs (Fig. 1) shows that MSB1, but not RP9, expresses $\alpha\beta$ TCR and CD3. These cell lines were subsequently used to isolate avian T-cell-specific genes by differential screening with subtracted probes. Radiolabeled, single-stranded cDNA prepared from polyadenylylated RNA from the T-LCL was hybridized to an excess of mRNA from the B-LCL. Double-stranded cDNA–mRNA hybrids were removed by hydroxylapatite chromatography, and the resulting probe (T-B), enriched for T-specific sequences, was hybridized to a first set of replicas of an MSB1 cDNA λgt10 library. The second set of replicas was hybridized to a control (B-B) probe, consisting of a population of labeled cDNA, generated from the B-LCL, that had been subtracted with its own mRNA template. Thus the two probes differed exclusively by the origins of the RNA templates, in order that a comparable plaque hybridization sensitivity was reached. After two rounds of screening, 27 cDNA clones were found to hybridize with the T-B probe but not with the B-B probe. The specificity of expression of these clones was assessed by hybridization to poly(A)$^+$ RNA from different tissues and cell lines. One clone (T11) detected a transcript of ≈1 kilobase (kb) in the MSB1 cell line as well as in several tissues known to contain T lymphocytes (i.e., spleen, thymus, and periph-

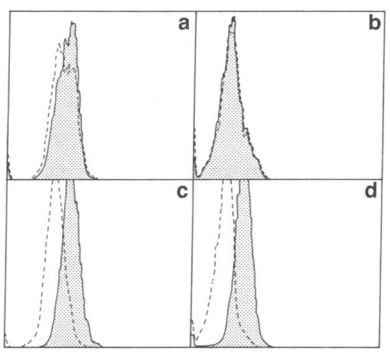

FIG. 1. Flow cytometric analysis of the RP9 and MSB1 LCLs. RP9 (*a*) and MSB1 (*c*) were stained with TCR2 and fluorescein isothiocyanate-conjugated F(ab')$_2$ goat anti-mouse immunoglobulin (——) or the conjugate alone (– – –). RP9 (*b*) and MSB1 (*d*) were stained with CT3 and fluorescein isothiocyanate-conjugated F(ab')$_2$ goat anti-mouse immunoglobulin (——) or second antibody alone (– – –).

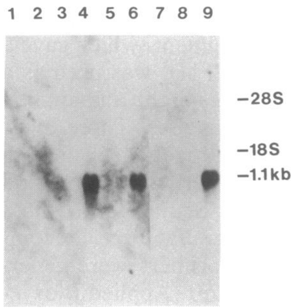

FIG. 2. Tissue-specific expression of T11.15. The T11.15 fragment was hybridized on a Northern blot of 5 μg of poly(A)+ RNA from mouse thymus (lane 1), chicken brain (lane 2), liver (lane 3), spleen (lane 4), bursa of Fabricius (lane 5) and thymus (lane 6), human T-LCL CEM (lane 7), and chicken LCLs RP9 (lane 8) and MSB1 (lane 9).

eral blood leukocytes). In contrast, no signal was detected with RNA from brain, liver, bursa of Fabricius, erythrocytes, the B-LCL, and the BM2C3 macrophage cell line (Fig. 2 and data not shown). Consequently, T11 expression is restricted to cells of the T lineage.

Nucleotide and Deduced Amino Acid Sequence of the Full-Length cDNA. The insert of clone T11 was used to screen a cDNA library constructed from the MSB1 LCL. Three positive overlapping clones were sequenced. The nucleotide sequence of the longest clone (T11.15) and its deduced amino acid sequence are shown in Fig. 3. T11.15 is 0.8 kb long and consists of a 5′ untranslated region of 31 base pairs (bp), an open reading frame of 525 bp, and a 241-bp-long 3′ untranslated region ending in a poly(A) tail 14 bp downstream of the consensus polyadenylylation signal ATTAAA (26). The putative ATG initiation codon, the first found in the sequence, lies downstream of an in-frame stop codon and is embedded in an initiation consensus sequence (27). The translated amino acid sequence yields a polypeptide of 175 amino acids with features of a type I transmembrane protein. The first methionine is followed by 24 predominantly hydrophobic residues resembling a signal peptide sequence (28). Cleavage of this peptide would yield a mature protein of 150 residues. Fifteen amino acids found by protein microsequencing of the chicken 19-kDa CD3 protein were encountered at this NH_2-terminal position (J. Lahti, C.-L. Kuo, R. Aebersold, L. Hood, and M. D. Cooper, personal communication). The molecular mass of the T11.15 polypeptide is 16,733 Da, which is consistent with the 16-kDa protein backbone of the 19-kDa chain of the CD3/TCR complex (13). Thus, the sequence data provide conclusive evidence that the T11.15 cDNA clone encompasses the complete coding information for this 19-kDa CD3 subunit. The extracellular 80-residue domain contains a potential N-linked glycosylation site (Asn-Ser-Thr; residues 63–65) and four cysteines. It is worth noting that the cysteine residues at positions 16 and 56 are organized in a manner characteristic of members of the immunoglobulin superfamily (10). This region is flanked by 26 predominantly hydrophobic amino acids, corresponding to the predicted transmembrane domain and contains a single negative charge (aspartic acid) at position 91. The predicted cytoplasmic region comprises 44 amino acids.

Homology with Mammalian CD3 Glycoproteins. By comparing T11.15 with the amino acid sequences of known proteins, the best matches were found with the human and murine CD3 δ and γ glycoproteins (29–32). The alignment shown in Fig. 4*a* indicates a perfect conservation of the four extracellular cysteines and a strong homology within and around the transmembrane domain. The predicted molecular masses of the mature protein backbones are similar (16–17 kDa). The sequence of the mature T11.15 protein shares 39.4% and 40.3% amino acid similarity with the murine and human δ chains and 36.3% and 38.7% with the murine and human γ chains, respectively. On the basis of the amino acid alignment, a hypothetical phylogenic tree traced according to a dendrogram of murine and human CD3 γ and δ, as well as chicken CD3 sequences, suggests that T11.15 branched before the duplication of the mammalian CD3 γ and δ genes (Fig. 4*b*).

Expression of the T11.15 Gene During Development. To determine when during embryonic development T11.15 is first expressed in thymocytes, this cDNA was used as a probe to detect the corresponding transcript in total RNA from thymus at various embryonic stages. Transcription of T11.15 mRNA was first detectable in thymuses of 10-day embryos

```
AAAAACACAGAGTTGCCATCACTCTGAGCC ATG TGG AAG GGA CGG GCC CTG GGC ACC TGG CTG CTG CTT  70
                               M   W   K   G   R   A   L   G   T   W   L   L   L
                               |               |
                              -25             -20

GCC TGC GTG GCT GTG GCC AAG TTG GGC GTC CAT GGA CTG AGC ATG AGT GTG AAA GAA GTC AGT GGG AAG G  140
 A   C   V   A   V   A   K   L   G   V   H   G   L   S   M   S   V   K   E   V   S   G   K
         |                                   |   |                                   |
        -10                                 -1  +1                                   10

TG TTC CTG CAA TGT CAA GAA AGC AAA GAT CTA AAC ACA AAT TAC CTT TGG AAG AAA GGT AAG GAG GAA TT  210
V   F   L   Q   C   Q   E   S   K   D   L   N   T   N   Y   L   W   K   K   G   K   E   E   L
                                |                                       |
                                20                                      30

A GGA AAC ATG AGG CAG CTG GAC TTG GGA GCA ATT TAC GAT GAT CCC AGA GGC ACC TAC ACA TGT CAG CGG  280
   G   N   M   R   Q   L   D   L   G   A   I   Y   D   D   P   R   G   T   Y   T   C   Q   R
                   |                                       |
                   40                                      50

GAT GAA AAC GTA AAC TCC ACT CTC CAC GTG CAC TAT CGA ATG TGC CAG AAT TGC ATT GAA GTG GAC GCT C  350
 D   E   N   V   N   S   T   L   H   V   H   Y   R   M   C   Q   N  ,C   I   E   V   D   A
     |                                   |                                       |
     60                                  70                                      80

CC ACC ATA TCA GGG ATT GTG GTC GCA GAT GTT GTC GCC ACT GTC TTG CTG GCA ATT GCT GTG TAT TGC AT  420
P   T   I   S   G   I   V   V   A   D   V   V   A   T   V   L   L   A   I   A   V   Y   C   I
                                |                                       |
                                90                                     100

C ACT GGG CAA GAT AAG GGA CTC ATG TCA CGA GCT TCT GAC AGG CAG AAC CTG ATA GCC AAC GAT CAG CTC  490
   T   G   Q   D   K   G   L   M   S   R   A   S   D   R   Q   N   L   I   A   N   D   Q   L
                   |                                       |
                  110                                     120

 TAC CAG CCC CTT GGT GAG CGG AAT GAT GGA CAG TAC AGC CAG CTG GCA ACT GCC AAG GCC CGC AAG TGAA  560
  Y   Q   P   L   G   E   R   N   D   G   Q   Y   S   Q   L   A   T   A   K   A   R   K   *
      |                                       |                                       |
     130                                     140                                     150

GTGGGAAGGCACTGGGGCCAGTGATTCCTTCACACGACGAAACCATAGCCAGTGCTAAAGAGCACCAGGACCCTGCCCTGGTGCTGTGTTGTG  654

AACTTGCTGCTGGCAGAAGACAGTGTGGATTTTTGCCTGGAAAGCCCTCCAGCTCCCTGCCAGGATGGGCTGCTTGTAGCACTCTGCTCTTTC  747

CATGATGCGTTGTATTTCTGTATTCCTCTCATTAAAGACGATGTTATTCT (A)n
```

FIG. 3. Nucleotide sequence of the T11.15 clone and its one-letter code translation. Numbering starts at the first nucleotide for the cDNA sequence and at the first amino acid of the mature protein for the polypeptide sequence. The putative glycosylation site and the polyadenylylation sequence are underlined and double-underlined, respectively. The stop codon is marked by an asterisk.

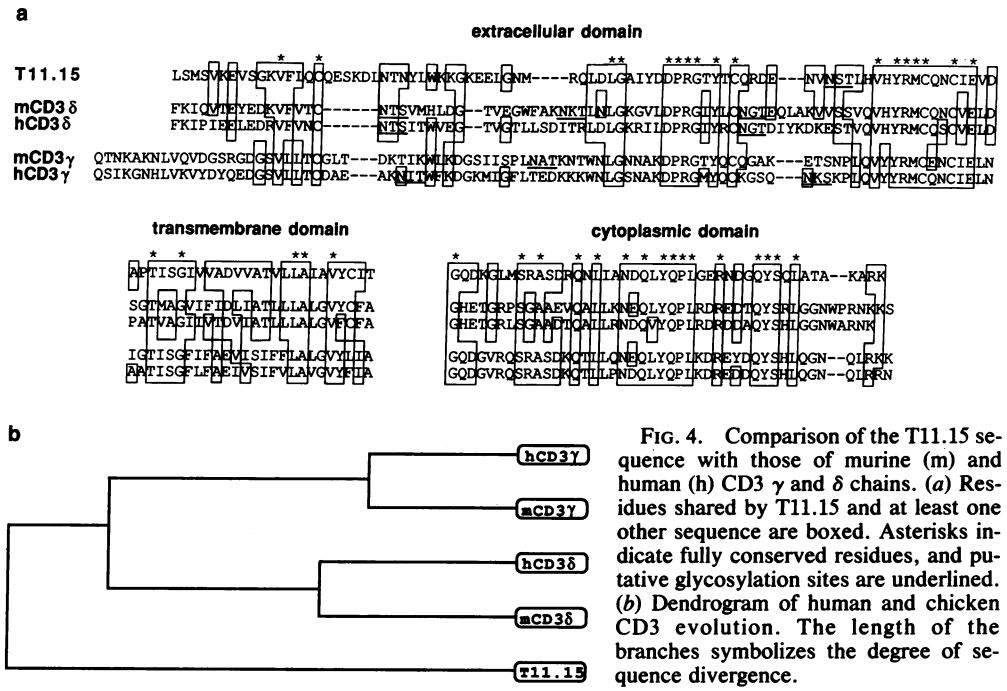

FIG. 4. Comparison of the T11.15 sequence with those of murine (m) and human (h) CD3 γ and δ chains. (*a*) Residues shared by T11.15 and at least one other sequence are boxed. Asterisks indicate fully conserved residues, and putative glycosylation sites are underlined. (*b*) Dendrogram of human and chicken CD3 evolution. The length of the branches symbolizes the degree of sequence divergence.

(Fig. 5). This signal increased until the early posthatching period. No T11.15 expression was found in thymuses of 6- to 9-day embryos.

DISCUSSION

Because mammalian probes specific for T-cell differentiation antigens fail to cross-hybridize with chicken DNA or mRNA (ref. 33; A.B., unpublished results), we used a combined strategy of subtractive hybridization and differential screening to isolate cDNA clones corresponding to genes preferentially expressed in chicken T cells. In this study, we report the characterization of a T-cell-specific cDNA encoding the 19-kDa CD3 glycoprotein of the chicken. Genomic DNA isolated from various chicken cell lines and tissues analyzed by Southern blot indicates that this chain is represented as a single-copy gene in the genome and that this gene does not rearrange in T-cell lines (data not shown).

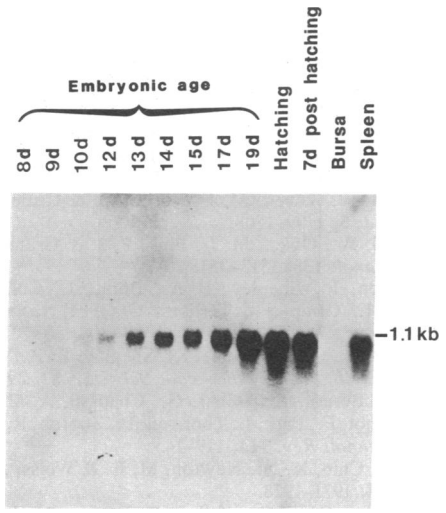

FIG. 5. Developmental expression of T11.15 mRNA in the thymus. The T11.15 fragment was hybridized on a Northern blot of 5 μg of total RNA from embryonic and early posthatching thymuses. The negative control was RNA from Bursa of Fabricius; the positive control was RNA from chicken spleen. d, Day.

Several interesting points arise from the comparison of the mammalian and avian CD3 sequences: in the extracellular domain, conservation concerns four cysteine residues and the portions Asp-Pro-Arg-Gly-Xaa-Tyr-Xaa-Cys (residues 49–56) and Val-Xaa-Tyr-Arg-Met-Cys-Xaa-Xaa-Cys-Xaa-Glu (residues 68–78), which are also conserved in the murine and human CD3 γ and δ chains (although in these latter, the stretches of conserved residues, Asp-Pro-Arg-Xaa-Tyr-Xaa-Cys-Xaa-Gly and Gln-Val-Xaa-Tyr-Arg-Met-Cys-Xaa-Xaa-Cys-Xaa-Glu-Leu, respectively, are slightly longer; ref. 30). Conservation over long phylogenic distances suggests that these residues have an important influence on the function and/or the tertiary conformation of these polypeptides. In contrast, the relative positions of the potential N-linked glycosylation sites are different. Both the chicken T11.15 and mammalian CD3 glycoproteins share the presence of a negatively charged amino acid in their transmembrane region. In mammalian CD3, this residue is thought to interact with a positive charge in the transmembrane region of the TCR, allowing the formation of a salt bridge between these chains (34, 35). An extra cysteine is also specifically shared by T11.15 and murine and human CD3 δ transmembrane domains. The cytoplasmic domain of this chicken subunit exhibits a high degree of similarity when compared with mammalian CD3 glycoprotein counterparts. Interestingly, the cytoplasmic segment Ser-Arg-Ala-Ser-Asp (residues 114–118) whose homologous sequence was postulated to be the phosphorylation site in the murine and human CD3 γ chains is also conserved in the cytoplasmic domain of T11.15. This suggests that the T11.15 cytoplasmic region could be phosphorylated upon activation of avian T lymphocytes.

Table 1 summarizes certain features emerging from this comparison. Whereas the extracellular and transmembrane regions of the chicken 19-kDa CD3 are more related to the mammalian CD3 δ counterparts, its cytoplasmic region is more homologous to the CD3 γ cytoplasmic regions. It was previously proposed by Krissansen *et al.* (30) that the mammalian CD3 δ and γ genes arose by duplication 230 million years ago—namely, after the separation of the mammalian and avian lineages (about 270 million years ago). This hypothesis is supported by the topology of the dendrogram calculated for CD3 δ and γ of mammalian origin and T11.15. At the present stage, we cannot exclude the possibility that

Table 1. Comparison between chicken and mammalian CD3 glycoproteins

	CD3 δ		19-kDa CD3	CD3 γ	
	Mouse	Human	chicken	Mouse	Human
Molecular mass (deglycosylated), kDa	16	16	17	16	16
Total no. of residues	152	150	150	160	160
No. of glycosylation sites	3	2	1	1	2
Charged amino acid in the transmembrane domain	Asp-90	Asp-90	Asp-91	Glu-100	Glu-100
Extra cysteine in the transmembrane region	Cys-103	Cys-103	Cys-104	—	—
% identical amino acids with T11.15					
Total polypeptide	39.4	40.3	—	36.3	38.7
Extracellular domain	36.8	35.6	—	27.4	29.5
Transmembrane domain	42.3	50.0	—	42.3	46.2
Cytoplasmic domain	42.6	43.5	—	51.1	53.2

both the 19-kDa and 20-kDa glycoproteins immunoprecipitated by the CT3 antibody (13) are derived from just one 17-kDa protein precursor by different posttranscriptional modifications. Nevertheless, it remains possible that a duplication occurred independently in the avian lineage, giving rise to analogs of mammalian CD3 γ and δ genes or that chicken CD3 γ and δ genes diverged before the avian/mammalian split and then evolved at different rates in the mammalian and avian lineages. To address this problem, the sequence of the chicken 20-kDa glycoprotein needs to be determined.

In Northern blot, T11.15 RNA expression is first detectable in thymocytes of 10-day chicken embryos. This contrasts with the appearance of immunoreactive $CD3^+$ thymocytes on day 9 (36). However, at this time, the antigen recognized by CT3 is restricted to the cytoplasm, and no cell surface expression occurs until day 12, when the first wave of $TCR1/CD3^+$ thymocytes is generated. This situation is reminiscent of the physiology of murine and human TCR, whose presence at the surface requires expression of the complete set of TCR/CD3 subunits. It is possible that the T11.15 chain does not bear the epitope recognized by the CT3 monoclonal antibody (which, like the majority of anti mammalian CD3 antibodies, could recognize the ε chain; ref. 37). If this were the case, our data could be integrated into the following hypothetical scenario of an ordered expression of TCR subunits during chicken thymocyte maturation. First, the nonglycosylated 17-kDa subunit, which carries the antigenic determinant recognized by CT3, is expressed at day 9. At day 10, the T11.15 gene is transcribed, giving rise to the 19-kDa (and possibly 20-kDa) CD3 glycoprotein. After a lag period of about 2 days, during which TCR1 (γδ) rearrangement and expression occur, all components of the CD3/TCR complex are available, thus allowing its assembly and surface expression.

We are indebted to Max Cooper (University of Alabama at Birmingham) for the gift of monoclonal antibodies CT3, TCR1, and TCR2, and for providing the unpublished amino-terminal sequence of the 17-kDa CT3-reactive subunit; Thomas Rhyner (Institut of Pharmacology, University of Zurich) for technical advice and helpful discussions; Pierre Vaigot for performing FACS analysis; Monique Coltey for dissection demonstrations; Guido Kroemer for critical reading of the manuscript; and Yann Rantier and Sophie Gourmet for illustrations. This work was supported by the Centre National de la Recherche Scientifique, and in part by grants from the Institut National de la Santé et de la Recherche Médicale, and the Association pour la Recherche sur le Cancer (to C.A.). A.B. is recipient of a fellowship from the Association pour la Recherche sur le Cancer.

1. Davis, M. M. & Bjorkman, P. J. (1988) *Nature (London)* **334**, 395–402.
2. Parnes, J. R. (1989) *Adv. Immunol.* **44**, 265–311.
3. Clevers, H., Alarcon, B., Wielman, T. & Terhorst, C. (1988) *Annu. Rev. Immunol.* **6**, 629–662.
4. Baniyash, M., Garcia-Morales, P., Bonifacino, J. S., Samelson, L. E. & Klausner, R. D. (1988) *J. Biol. Chem.* **263**, 9874–9878.
5. Weiss, A., Imboden, J., Hardy, K., Manger, B., Terhorst, C. & Stobo, J. (1986) *Annu. Rev. Immunol.* **4**, 593–619.
6. Alexander, D. R. & Cantrell, D. A. (1989) *Immunol. Today* **10**, 200–204.
7. Weissman, A. M., Baniyash, M., Hou, D., Samelson, L. E., Burgess, W. H. & Klausner, R. D. (1988) *Science* **239**, 1018–1021.
8. Weissman, A. M., Hou, D., Orloff, D. G., Modi, W. S., Seuanez, H., O'Brien, S. J. & Klausner, R. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9709–9713.
9. Jin, Y. J., Clayton, L. K., Howard, F. D., Koyasu, S., Sieh, M., Steinbrich, R., Tarr, G. E. & Reinherz, E. L. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3319–3323.
10. Williams, A. F. & Barclay, A. N. (1988) *Annu. Rev. Immunol.* **6**, 381–406.
11. Sowder, J. T., Chen, C. L. H., Ager, L. L., Chan, M. M. & Cooper, M. D. (1988) *J. Exp. Med.* **167**, 315–322.
12. Chen, C. L. H., Cihak, J., Lösch, U. & Cooper, M. D. (1988) *Eur. J. Immunol.* **18**, 539–543.
13. Chen, C. L. H., Ager, L. L., Gartland, G. L. & Cooper, M. D. (1986) *J. Exp. Med.* **164**, 375–380.
14. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
15. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
16. Rhyner, T. A., Faucon-Biguet, N., Berrard, S., Borbély, A. A. & Mallet, J. (1986) *J. Neurosci. Res.* **16**, 167–181.
17. Britten, R. J., Graham, D. E. & Neufeld, B. R. (1974) *Methods Enzymol.* **29E**, 363–406.
18. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
19. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd ed.
20. Seed, B. (1987) *Nature (London)* **329**, 840–842.
21. Dower, W. J., Miller, J. F. & Ragsdale, C. W. (1988) *Nucleic Acids Res.* **16**, 6127–6145.
22. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
23. Kanehisa, M. I. (1984) *Nucleic Acids Res.* **12**, 203–213.
24. Higgins, D. G. & Sharp, P. M. (1988) *Gene* **73**, 237–244.
25. Felsenstein, J. (1988) *Annu. Rev. Genet.* **22**, 521–565.
26. Birnstiel, M. L., Busslinger, M. & Strub, K. (1985) *Cell* **41**, 349–359.
27. Kozak, M. (1984) *Nucleic Acids Res.* **12**, 857–872.
28. Von Heijne, G. (1986) *Nucleic Acids Res.* **14**, 4683–4690.
29. Krissansen, G. W., Owen, M. J., Verbi, W. & Crumpton, M. J. (1986) *EMBO J.* **5**, 1799–1808.
30. Krissansen, G. W., Owen, M. J., Fink, P. J. & Crumpton, M. J. (1987) *J. Immunol.* **138**, 3513–3518.
31. Van den Elsen, P., Shepley, B. A., Borst, J., Coligan, J. E., Markham, A. F., Orkin, S. & Terhorst, C. (1984) *Nature (London)* **312**, 413–418.
32. Van den Elsen, P., Georgopoulos, K., Shepley, B. A., Orkin, S. & Terhorst, C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2944–2948.
33. Kroemer, G., Bernot, A., Béhar, G., Chaussé, A. M., Gastinel, L. N., Guillemot, F., Park, I., Thoraval, P., Zoorob, R. & Auffray, C. (1990) *Immunol. Rev.* **113**, 119–145.
34. Morley, B. J., Chin, K. N., Newton, M. E. & Weiss, A. (1988) *J. Exp. Med.* **168**, 1971–1978.
35. John, S., Banting, G. S., Goodfellow, P. & Owen, M. J. (1989) *Eur. J. Immunol.* **19**, 335–339.
36. Bucy, R. P., Chen, C. L. H. & Cooper, M. D. (1988) *J. Immunol.* **144**, 1161–1168.
37. Transy, C., Moingeon, P. E., Marshall, B., Stebbins, C. & Reinherz, E. L. (1989) *Eur. J. Immunol.* **19**, 947–950.