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Evidence for a Stepwise Evolution of the CD3 Family

Thomas W. F. Göbel2* and Jean-Pierre Dangy†

The three CD3 components of the TCR complex are encoded as clustered genes in mammals. The evolution of such a multimeric complex is likely to occur stepwise. The chicken CD3 cluster was entirely sequenced, and, in contrast to mammals, only two chicken CD3 genes were found to be physically linked to the unrelated genes HZW10 and epithelial V-like Ag flanking both sides of the CD3 cluster. Biochemical analyses of CD3 immunoprecipitates confirmed the presence of only two CD3 proteins and revealed an essential role for CD3γδ glycosylation during assembly. Functional analyses indicated that the chicken TCR/CD3 complex was efficiently down-regulated by phorbol ester treatment, demonstrating the integrity of a CD3γ-like cytoplasmic internalization motif. These data argue for a stepwise CD3 evolution, with major differences in the TCR/CD3 structure between mammalian and nonmammalian vertebrates setting a basis for the understanding of the CD3 phylogeny and proving the ancestral nature of the CD3γδ protein. The Journal of Immunology, 2000, 164: 879–883.

Mammalian TCR/CD3 proteins have been the focus of intense research covering aspects of biosynthesis, assembly, structure, and signaling in the past few years (1, 2). The clonotypic TCR chains have been characterized in a number of different vertebrate phyla (3). In contrast, the difficulties in cloning of nonmammalian CD3 homologues has limited their detailed analysis. The chicken TCR/CD3 represents the only nonmammalian Ag receptor where two CD3 genes and the ϶-chain have been identified (4–6). Since data regarding the CD3 biochemistry does not exclude the presence of a third CD3 (7), the present experiments were performed to reveal the actual complexity of the avian TCR.

The CD3 genes are clustered on a 50-kb part of chromosome 11 and 9 in humans and mouse, respectively. CD3δ is located in the central position of the cluster and flanked on either side by the oppositely transcribed CD3γ and CD3ϵ gene loci. CD3γ is less than 2 kb apart from CD3δ, and CD3ϵ is located about 22 kb downstream from CD3δ (8). CD3δ is encoded by five exons, whereas seven exons encode CD3γ and seven or eight exons encode CD3ε depending on the presence of two and three miniexons in humans and mouse, respectively. All CD3 genes have TATA-less, non-tissue-specific promoters, and enhancer elements to ensure T cell-specific expression (9–12).

To date, only three nonmammalian CD3 homologues and the chicken ϶-chain have been identified (4–6, 13). The chicken CD3ϵ chain has low extracellular (EX)3 but high cytoplasmic (CY) homology to its mammalian counterparts (5). An additional CD3 chain has been cloned in the chicken and in the amphibian Xenopus laevis. It shares equal homology to both mammalian CD3γ and CD3δ, and it was therefore designated CD3γ6 (4, 13). Calculations based on sequence divergence further demonstrated that human and mouse CD3γ and CD3δ originate from a gene duplication which occurred about 230 million years ago (14). Since birds and mammals are separated by about 250 million years, this gene duplication could have occurred after the lineage separation.

A recent gene duplication could imply a redundancy of the duplicated genes. This holds true for certain CD3 domains. It has been shown that the CD3γ transmembrane (TM) domain can be replaced by that of CD3δ. In contrast, domain swapping of the CD3γ and CD3δ EX domains causes loss of TCR surface expression (15). The CD3γ and CD3δ CY domains are not essential for surface expression (16, 17). CD3γ harbors a unique di-leucine based internalization motif mediating protein kinase C-dependent TCR (18). Therefore, phorbol ester-mediated TCR down-regulation is abrogated in T cells with CY-truncated CD3γ and chimeric CD3γ with a CD3δ CY domain, whereas anti-CD3-mediated TCR down-regulation is still intact (15, 17).

In contrast, gene targeting of individual CD3 genes has clearly established unique nonredundant functions during T cell development for both CD3γ and CD3δ. The CD3γ chain is essential for a functional pre-TCR since thymocyte development is blocked before β selection in the double-negative CD4−CD25+ stage, whereas thymocyte development in the CD3δ knockout is arrested at the double-positive stage. Moreover, γδ T cells do develop in the absence of CD3δ, but are virtually absent in the CD3γ knockout (19, 20).

For the first time, we now report the complete sequence and physical linkage of the entire CD3 cluster. These analyses have failed to identify a third CD3 gene in the chicken. In addition, biochemical and functional analyses establish the ancestral hybrid nature of the chicken CD3γδ protein. These results raise the challenging question why a third CD3 protein evolved as an additional essential component of the mammalian TCR complex.

Materials and Methods
Isolation and sequencing of cosmid clones
A commercial chicken cosmid library (Clontech, Palo Alto, CA) was hybridized with a full-length CD3ε cDNA probe, and two reactive cosmids were isolated. Cosmid DNA was prepared (Qiagen, Hilden, Germany) and directly used as template for sequencing using CD3-specific and internal oligonucleotides with the ABI Prism dye terminator cycle sequencing...
The human and mouse CD3 genes are flanked on both sides by chicken repeat 1 elements (22). The chicken CD3 locus is smaller than in humans or mouse, spanning only 9.5 kb as opposed to 50 kb in humans. The different CD3 proteins were also identified in parallel Western blot experiments using chain-specific antisera on another chromosome. To exclude this possibility, the CD3 genes with other unrelated genes.

Two CD3 proteins are associated with the chicken TCR heterodimer

Although unlikely, our linkage analysis did not exclude the existence of a third CD3 gene encoded outside of the cluster or even on another chromosome. To exclude this possibility, the CD3 genes were metabolically labeled and immunoprecipitated with the CT3 mAb under mild conditions, preserving the TCR/CD3 interaction. Initially, the immunoprecipitates were analyzed in a one-dimensional gel comparing glycosylated and deglycosylated samples (Fig. 2A). The different CD3 proteins were also identified in parallel Western blot experiments using chain-specific antisera (data not shown). The TCR-γ/δ migrated around 70 kDa and dropped to 50 kDa following deglycosylation. Additional free

RESULTS

The chicken CD3 cluster contains only two CD3 genes flanked by unrelated genes

To test whether a third CD3 gene exists in the chicken CD3 locus, we sequenced the entire CD3 cluster (Fig. 1; accession number AJ250458). The chicken CD3 locus is smaller than in humans or mouse, spanning only 9.5 kb as opposed to 50 kb in humans. The oppositely transcribed CD3γδ and CD3ε genes are only 2 kb apart instead of the 22 kb distance in humans (Fig. 1). The two CD3 genes are flanked on both sides by chicken repeat 1 elements (22). The human and mouse CD3γ genes are located less than 2 kb upstream of CD3δ (Fig. 1A). In the chicken, however, a highly conserved centromere/kinetochore protein (HZW10) (23) is present in the place of CD3γ, only 832 bp upstream of CD3γδ. Moreover, the chicken homologue of the recently described epithelial V-like Ag (24) is located 4.2 kb downstream of CD3ε on the other side of the CD3 cluster (Fig. 1B). To our knowledge, the human CD3 cluster has not yet been physically linked to other genes; however, both epithelial V-like Ag and HZW10 genes have also been mapped to human chromosomes 11q23 and 11q24, respectively.

The CD3γδ gene consists of five exons with identical splicing boundaries as mammalian CD3δ. The chicken CD3ε is encoded by seven exons. It includes one miniegion encoding seven amino acids, whereas two and three miniegions have been described for mouse and human CD3ε, respectively. Both chicken CD3γδ and CD3ε lack promoters with TATA or CCAAT elements, and the 2-kb spacer contains multiple potential transcription factor binding sites with critical roles in regulating T cell-specific gene expression like TCF-1, GATA-3, and IKAROS. This genomic organization demonstrates the absence of a third CD3 gene within the CD3 cluster and for the first time physically links the chicken CD3 genes with other unrelated genes.

While the molecular analysis did not exclude the existence of a third CD3 gene encoded outside of the cluster or even on another chromosome. To exclude this possibility, the CD3 genes were metabolically labeled and immunoprecipitated with the CT3 mAb under mild conditions, preserving the TCR/CD3 interaction. Initially, the immunoprecipitates were analyzed in a one-dimensional gel comparing glycosylated and deglycosylated samples (Fig. 2A). The different CD3 proteins were also identified in parallel Western blot experiments using chain-specific antisera (data not shown). The TCR-ε/δ migrated around 70 kDa and dropped to 50 kDa following deglycosylation. Additional free

FIGURE 1. Genomic organization of the human and chicken CD3 cluster. A, Sketch representing the human CD3 cluster organization (8, 9, 12). Approximate sizes of the CD3 genes and the intervening sequences are given. Note that the sketch does not represent the exact size. B, Genomic organization of the chicken CD3 cluster and its derived mRNA drawn to scale as indicated. The genomic DNA and mRNA are represented in the top and bottom, respectively. Exons are shown as closed boxes and the chicken repeat (CR1) elements as hatched boxes. EVA, epithelial V-like Ag; SP, signal peptide.

For metabolic labeling, 50 million UG9 cells were incubated for 3 h in the presence of 500 μCi [35S]methionine/cysteine (Promix; Amersham, Piscataway, NJ) and when indicated in the presence of 1 μg/ml tunicamycin (Boehringer Mannheim, Indianapolis, IN), washed, and lysed with 1% digitonin in 300 mM NaCl, 100 mM Tris-Cl (pH 7.4), 10 mM EDTA, 0.2% NaN3, 40 mM iodoacetamide, and protease inhibitors (Complete; Boehringer Mannheim). Deglycosylation was performed using PNGase F (New England Biolabs, Beverly, MA). Immunoprecipitations were performed with the CT3 mAb. The immunoprecipitates were either directly separated by 4–20% PAGE or first on a NEPHGE tube gel followed by 9–18% PAGE.

Immunoprecipitation, SDS-PAGE, and nonequilibrium pH gradient electrophoresis (NEPHGE)

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The chicken ab T cell line UG9 was maintained in DMEM supplemented with 5% FCS and 1 mM glutamine. The cells were incubated with the indicated amounts of phorbol 12,13-dibutyrate (PDB; Sigma, St. Louis, MO) for 1 h at 37°C and then collected and labeled using either the chicken CD3ε-specific CT3 mAb or the chicken αVβ1-specific TCR2 mAb (Southern Biotechnology Associates, Birmingham, AL) (7, 21), followed by goat anti-mouse IgG1-FITC conjugate (Southern Biotechnology Associates). The flow cytometric analysis was performed on a FACScan (Becton Dickinson, Mountain View, CA), and the percentage of TCR down-regulation was calculated [mean fluorescence intensity of PDB-treated cells/mean fluorescence intensity of untreated cells × 100].

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TCRβ-chain was detected at 45 kDa and 29 kDa following deglycosylation. Single CD3 proteins were identified as the glycosylated 19-kDa CD3γδ protein and the nonglycosylated 16-kDa CD3ε protein. After deglycosylation, the CD3γδ and CD3ε proteins were not separated well on a 4–20% PAGE and comigrated. In addition, both CD3 proteins formed a covalently linked CD3γδ-CD3ε heterodimer of 35 kDa that migrated faster following PNGase F treatment due to the presence of N-linked carbohydrates in CD3γδ.

To further improve the resolution, NEPHGE analysis was employed on metabolically labeled, PNGase F-treated CT3 mAb immunoprecipitates (Fig. 2B). This analysis revealed multiple forms of the TCR-αβ heterodimer migrating around 50 kDa which were associated with two CD3 proteins resembling the acidic CD3ε monomer, the basic CD3γδ protein, and the CD3γδ-CD3ε heterodimer with intermediate charge (Fig. 2B). The monomeric TCRβ-chain was detected as basic protein close to the CD3γδ-CD3ε heterodimer. Two proteins migrating at about 36 and 38 kDa were identified as the ζ-ε-homodimer due to their migration at the basic end of the gel and their protein core of 17 kDa following reduction (Fig. 2B and data not shown).

An important biochemical difference between mammalian CD3γ and CD3δ is the dependence of glycosylation for TCR assembly. Although glycosylation of CD3δ is essential for TCR assembly, CD3γ glycosylation is dispensable (16). To test whether the glycosylation of the chicken CD3γδ protein is important for the assembly of the TCR complex, the T cell line was metabolically labeled in the presence of tunicamycin to prevent N-linked glycosylation. Immunoprecipitation with CD3ε-specific mAb revealed no association with CD3γδ or TCR. Apparently, CD3γδ glycosylation is required for the assembly with CD3ε and TCR and the transport to the cell surface (Fig. 3). Interestingly, in the absence of CD3γδ association, the CD3ε chain formed dimers, as has been reported for mammalian CD3ε (Fig. 3) (25). Mammalian CD3γ and CD3δ proteins drastically differ in their susceptibility to CY degradation (1, 2). When the stability of the chicken CD3 proteins was tested in pulse-chase experiments, both CD3 proteins were stable for several hours like mammalian CD3γ, but in contrast to mammalian

FIGURE 2. Metabolic analyses of the chicken TCR/CD3 complex. A, Metabolically labeled UG9 T cells were lysed in 1% digitonin and immunoprecipitated with a CD3ε-reactive mAb and either directly analyzed by 4–20% gradient PAGE or treated with PNGase F to remove N-linked carbohydrates. The identity is indicated for the glycosylated proteins and changed following deglycosylation as described in Results. B, Immunoprecipitates were deglycosylated with PNGase F and separated by NEPHGE followed by PAGE on a 9–18% gradient gel. The identity of the proteins is indicated.

FIGURE 3. Glycosylation of CD3γδ is essential for the TCR/CD3 assembly. UG9 cells were metabolically labeled in the absence or presence of tunicamycin to prevent N-linked glycosylation as indicated. Immunoprecipitations were done with the CT3 mAb (+) or an isotype control mAb (–). The molecular identity of the proteins and the apparent Mr is indicated.

FIGURE 4. PDB treatment induces TCR down-regulation. The percentage of TCR/CD3 down-regulation of UG9 cells incubated with increasing concentrations of PDB for 1 h at 37°C as analyzed by CD3 (■) and TCR surface staining (□). Means ± SD of triplicates are shown.
CD3δ which is rapidly degraded (data not show). Taken together, these experiments biochemically prove the existence of only two nonmammalian CD3 proteins, a CD3δ homologue and a second CD3γδ protein with hybrid character combining features of mammalian CD3γ and CD3δ.

**TCR down-regulation of the chicken TCR**

The chicken CD3γδ protein shares many characteristics with mammalian CD3δ (see below). However, a highly conserved CD3γ-like feature of the CD3γδ chain is the presence of an internalization motif within the CY domain, which mediates protein kinase C-dependent TCR down-regulation (15). To demonstrate that this motif has been functionally conserved, the UC9 T cell line was incubated with increasing amounts of PDB, and the degree of TCR down-regulation was determined by CD3ε or TCRβ staining and analyzed by flow cytometry. Within 1 h of incubation, the TCR was down-regulated by PDB treatment in a dose-dependent fashion (Fig. 4). These results demonstrate that the CY CD3γ-like internalization motif in the CD3γδ protein is fully intact.

**Discussion**

This report presents conclusive evidence for the absence of a third CD3 gene and CD3 protein in the chicken and most likely all nonmammalian vertebrates. The absence of a third CD3 protein is supported by 1) lack of a third CD3 gene at the expected genomic position, 2) lack of biochemical evidence for a third CD3 protein, 3) theoretical predictions dating the CD3 duplication at about 230 million years (14), and 4) the hybrid character of both the chicken and X. laevis CD3γδ protein (see below, Table I).

The biochemical analysis of the chicken TCR/CD3 complex has complicated the issue regarding two or three CD3 chains, since surface iodination consistently reveals three protein bands of 20 kDa, 19 kDa, and 17 kDa. The 20-kDa and 19-kDa glycoproteins, however, represent differentially glycosylated forms of the same protein, because they share a protein core size of 17 kDa upon deglycosylation (Ref. 7) and T W. F. Göbel, unpublished observation) and an identical N-terminal peptide sequence (data not shown). The metabolic analyses presented here suggest the presence of only two CD3 proteins. Glycosylation of CD3γδ but not CD3ε helped to distinguish both proteins since their protein core sizes are nearly identical (16.7 kDa). In addition to the monomeric forms, both CD3 proteins formed a covalently linked heterodimer, which we have consistently observed in metabolic labeling studies and in vitro expression experiments (Fig. 2A and T. W. F. Göbel, unpublished observation). The role of this heterodimer during assembly and/or surface expression is currently unclear. The biochemical analyses presented here correspond to and add information to the previously published analyses of iodinated chicken lymphocytes (7). A discrepancy regarding the migration of the deglycosylated CD3 proteins most likely reflects the difficulty in separating these two closely related proteins under different experimental conditions.

The experimental evidence presented here shows that the CD3γδ gene resembles an ancestral form that combines typical features of mammalian CD3γ and CD3δ, which are critically relevant for proper TCR assembly and function (Table I). To substantiate this hypothesis, all mammalian CD3γ and CD3δ chains were aligned with their two nonmammalian homologues to pinpoint diagnostic residues that are only present in mammalian CD3γ and the nonmammalian CD3γδ chains, but not CD3δ and vice versa. The EM domains contain few conserved features, including the four cysteines present in all CD3 proteins and the DPRG motif known to create a binding site for CD3ε (16). The unique CXXCXE motif in the membrane proximal region present in all CD3 chains is followed by a second negatively charged aspartic acid in mammalian CD3δ and CD3γδ, but not CD3γ (Table I). Moreover, the glycosylation of CD3γδ is essential for its assembly with CD3ε as in mammalian CD3δ, whereas CD3γ glycosylation is dispensable (Fig. 3). The negatively charged TM residues represent an aspartic acid in CD3γδ as in the mammalian CD3δ proteins, whereas CD3γ proteins harbor a glutamic acid at this position. All of these criteria and also the five exon genomic structures and the genomic location would suggest that the chicken CD3γδ closely resembles a CD3δ homologue. However, its CY domain harbors a CD3γ-like internalization motif. It was therefore critical to demonstrate that this internalization motif in CD3γδ is functionally active in chicken T cells (Fig. 4). The conserved change of the chicken motif from a leucine to an isoleucine does not affect this function as has been demonstrated by previous mutational studies of human CD3γ (26). Moreover, unlike mammalian CD3δ, the chicken CD3γδ protein is slowly degraded. In conclusion, the chicken CD3γδ protein resembles mammalian CD3δ in most aspects, with the exception of the CY internalization motif and its slow degradation which are CD3γ-like features.

The five exon CD3δ structures most likely resemble the most primordial form of a CD3 gene, since the basic organization is found in every CD3 gene with variations occurring only at the ends of the CD3 genes. Based on the genomic organization presented here, a model for the CD3 evolution would predict two successive gene duplications where a single CD3 gene first duplicated to form CD3γδ and CD3ε and a second duplication of CD3γδ has finally generated mammalian CD3γ and CD3δ. Several features argue in favor of such a hypothesis: 1) the central location of CD3δ flanked by CD3γ and CD3ε on either side with opposite transcriptional orientations (Fig. 1) (8, 9, 12), 2) the TATA-less, non-tissue-specific promoters of all CD3 genes, 3) the conserved EX CXXC motif close to an exon-intron boundary, 4) the CY ITAM motif consistently interrupted by a type 0 intron, and 5) the 5′ exons present in all CD3 genes with variations occurring only at the 5′ and 3′ ends.

All TCR chains cloned to date contain a short CY domain lacking intrinsic enzymatic or signaling activity as well as a positively charged TM residues. It is therefore likely that the TCR heterodimer of lower vertebrates also associate with signal-transducing elements. It should be possible to find an even more rudimentary situation before the initial CD3 gene duplication. In striking difference to the proposed decameric mammalian TCR, where two TCR heterodimers associate with the ζζ-homodimer and two CD3 heterodimers, such a primordial receptor could have a pentameric structure consisting of a single TCR heterodimer, ζζ-homodimer, and single CD3 chain.

The model proposed for the chicken TCR/CD3 complex closely resembles the decameric model for the mammalian TCR, with the exception that the mammalian CD3γ-CD3ε heterodimer would be
replaced by a second CD3γδ-CD3ε heterodimer. It is interesting to note that at least two forms of mammalian TCR complexes are not affected by a loss of the CD3δ, namely, the pre-TCR and the TCR complex on γδ T cells, as demonstrated by the gene targeting of the CD3δ chain (19). In this aspect, these TCR complexes might represent a rudimentary form of an ancestral TCR and provide a paradigm where the ontogeny reflects the phylogeny of a cell surface receptor.

The most important question provoked by these results is why an already complex cell surface receptor with apparently normal function was further modified by the addition of a novel essential protein. An additional protein in the complex may be important for the fine tuning of the response by generating new binding sites for coreceptors. It may also change the entire three-dimensional structure of the receptor, thus influencing the MHC/peptide binding. An additional CD3 protein may also alter the TCR/CD3-signaling capacity. It is intriguing to note the correlation of a solvent exposed loop in the TCRβ-chain located between the V and C region only present in mammals and the occurrence of a third CD3 protein. Thus, this loop may create an important binding site for the CD3γ complex. The future analyses of these primordial TCR/CD3 complexes will contribute to the understanding of the TCR complex evolution and function.

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References


