Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7108–7112, September 1989
Immunology

Deletion of the cytoplasmic region of the CD3ε subunit does not prevent assembly of a functional T-cell receptor

CATHERINE TRANSY*†, PHILIPPE MOINGEON*†, CHRISTOPHER STEBBINS*, AND ELLIS L. REINHERZ*‡

*Laboratory of Immunobiology, Dana–Farber Cancer Institute; and Departments of †Pathology and ‡Medicine, Harvard Medical School, Boston, MA 02115

Communicated by H. Sherwood Lawrence, May 25, 1989 (received for review February 17, 1989)

ABSTRACT **The T-cell receptor (TCR) is a molecular complex comprised of a clonally restricted, immunoglobulin-like heterodimer (Ti), responsible for specific antigen recognition, and a set of monomorphic polypeptide CD3 subunits, termed γ, δ, ε, ζ, and η, presumed to be involved in transmembrane signaling events. To investigate the role of the CD3ε subunit in signal transduction, we have transfected a murine hybridoma T-cell line with either wild-type or variant human CD3ε cDNA that encodes a protein lacking 49 of the 55 cytoplasmic amino acid residues. Both wild-type and truncated CD3ε human proteins assemble with endogenous murine CD3/Ti subunits to form functional surface TCRs: Anti-human CD3ε monoclonal antibodies bind exclusively to these chimeric TCRs and trigger interleukin 2 production from the murine cells. Thus, the CD3ε cytoplasmic domain is not required for assembly of the multimeric TCR. Furthermore, it is dispensable for the transduction of a stimulus delivered to the external part of the molecule, suggesting that interaction between the transmembrane and/or external regions of the other TCR chains is a prerequisite for transmembrane signaling.**

The T-cell receptor (TCR) is a molecular complex comprised of a clonally restricted immunoglobulin-like heterodimer (Ti), responsible for specific major histocompatibility complex (MHC)-restricted antigen recognition and a set of invariant CD3 subunits, termed γ, δ, ε, ζ, and η (1–5). The function of CD3 polypeptides remains to be elucidated but it has been proposed that the CD3 components are directly involved in the transmembrane signaling events. The following points support this hypothesis: (*i*) Unlike Ti elements, CD3 subunits have substantial cytoplasmic domains (6–9), several of which undergo phosphorylations upon T-cell stimulation (10, 11); and (*ii*) the binding of anti-CD3 monoclonal antibodies (mAbs) to the T-cell surface mimics the antigen interaction with the TCR since it triggers early events (elevation of cytoplasmic-free calcium and inositol phospholipid breakdown) as well as late events of T-cell activation (lymphokine secretion and cell proliferation; for review, see ref. 12).

The elucidation of mechanisms that govern signal transduction through the TCR will require the function–structure analysis of individual CD3 subunits. Such analysis is complicated because an individual CD3 subunit is expressed at the plasma membrane only within the assembled TCR complex (13). Mutational analysis and reconstitution studies provide a powerful methodology to study gene function. The isolation of human T-cell variants deficient in an individual CD3 subunit is, however, difficult since allelic exclusion operates at the level of expression of the Ti but not CD3 subunits and strongly biases the frequency of surface $CD3^-$ cells (selected by strategies employing anti-CD3 or anti-Ti mAbs) in favor of Ti^- mutants. A strategy of interspecies complementation (14, 15) between subunits of the CD3–Ti complex represents an alternative means to examine the function of the CD3 elements when mAb probes are available to distinguish a given endogenous from exogenous subunit and permit selective stimulation of a chimeric receptor. Such is the case for analysis for the CD3ε subunit, which is examined in the present study.

MATERIALS AND METHODS

Cell Lines and mAbs. The 3DO54.8 cell line, a murine T–T hybridoma cell line specific for ovalbumin in the context of $I\text{-}A^d$ class II MHC antigen, and the murine B-cell line A20 used as antigen-presenting cells have been described by Marrack *et al.* (16) and were provided by P. Marrack (National Jewish Hospital, Denver). The interleukin 2 (IL-2)-dependent murine T-cell line (CTLL20) used for conventional IL-2 bioassays was provided by K. Smith (Dartmouth Medical School, Hanover, NH). Vit3b (provided by W. Knapp, Vienna) and Leu4 (provided by R. Evans, Sloan Kettering, New York) are murine IgG1 mAbs that bind to the human CD3ε subunit (17). RW2-8C8 is a murine anti-human CD3 IgG1 (1). 145.2C11 (2C11) is an anti-murine CD3ε IgG produced by a hamster–mouse somatic hybrid cell line (18). Vit3b, Leu4, and RW2-8C8 were grown as ascites fluid and 2C11 was obtained from culture supernatant. All mAbs were affinity-purified over protein A using the MAPS II kit from Bio-Rad.

Isolation of a cDNA Clone Coding for the Human CD3ε Subunit and Construction of the Mutant CD3εΔC. We used a labeled antisense oligonucleotide corresponding to positions 111–152 of the published nucleotide sequence of a human CD3ε cDNA (8) to screen a cDNA library constructed in vector pcDV1 from poly(A)$^+$ RNA from a human T-cell clone (19). Determination of the nucleotide sequence of the positive clone pcDVε confirmed that it contained a complete coding sequence for the human CD3ε subunit. In the 5′ noncoding region, pcDVε contained the additional sequence GAGAGAAAAATGAAGTA upstream of the first nucleotide in the published sequence of the CD3ε cDNA (8). The 1400-base-pair (bp) *Bam*HI fragment of pcDVε, which contains the complete CD3ε cDNA, was used for subsequent subclonings. For mutagenesis, the CD3ε cDNA subcloned into the PGEM4 vector (Promega) was linearized using the unique *Sty* I site. The *Sty* I site was made blunt using the Klenow fragment of DNA polymerase I and the linearized plasmid was ligated with the nonphosphorylated DNA linker 5′-CTAGTCTAGACTAG-3′ (New England Biolabs) present in a 100-fold molar excess. During the process of purification of the ligated DNA from low-melting-point agarose gel, noncovalently bound single-stranded oligonucleotides were eliminated leaving single-stranded cohesive ends. After reannealing, the noncovalently closed DNA was directly used to transform *Escherichia coli*. Insertion of the oligonucleotide was assessed by digestion with *Xba* I (the linker contains an

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Abbreviations: TCR, T-cell receptor; mAb, monoclonal antibody; IL-2, interleukin 2; MHC, major histocompatibility complex.

Xba I site) and confirmation of the expected modification was obtained by direct nucleotide sequencing.

Infection with Recombinant Retrovirus and Protoplast Fusion. The CD3ε or the CD3εΔC insert was subcloned using the unique *Bam*HI site of the recombinant retrovirus vector DOL. In the resulting DOLε or DOLεΔC plasmids, the neomycin-resistance gene is controlled by the simian virus 40 promoter whereas the CD3ε cDNA transcription is driven by the murine leukemia virus long terminal repeat. DOLε or DOLεΔC plasmid DNAs were transfected into the retrovirus-packaging ψ2 cell line to obtain permanent producers of recombinant retrovirus. Infections were carried out by incubating 10^5 3DO54.8 cells for 18–24 hr with diluted retrovirus stock in the presence of Polybrene (8 μg/ml; Aldrich). The infected cell population was distributed into 24-well plates at 5×10^3 cells per well and selection using G418 (0.5 mg/ml; GIBCO) was initiated 48 hr after infection. G418-resistant clones were further maintained in complete medium containing G418 (0.5 mg/ml).

For protoplast fusion, the CD3ε or the CD3εΔC cDNAs were inserted at the unique *Bam*HI site of the pPink.2 vector (kindly provided by P. S. Ohashi, University of Toronto). In the resulting Pinkε or PinkεΔC plasmids, the cDNA insert and the neomycin-resistance gene expressions are driven by the spleen focus-forming virus long terminal repeat and promoter, respectively. The Pinkε and PinkεΔC were grown in *E.coli* strain HB101 and amplified using chloramphenicol. The cultures were then converted to protoplasts and fused to the 3D054.8 cells as described (20). Cells were plated in 24-well plates and selection was as above. G418-resistant clones were maintained in complete medium containing G418 (0.5 mg/ml).

Southern Blot Analysis. Genomic DNA was prepared from cell lines. DNA (10 μg) was digested using 40 units of each restriction enzyme (New England Biolabs). After electrophoresis in 1% agarose gel, the digested DNA was blotted onto Zeta-bind nylon membrane (Bio-Rad) according to the manufacturer's recommendations. After hybridization with the ^{32}P-labeled CD3ε cDNA insert, the Southern blot was washed for two 30-min periods in 0.1× SSC/0.1% SDS at 55°C and autoradiographed for 18 hr (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0).

Immunoprecipitation. Cell surface labeling of the cells with ^{125}I using lactoperoxidase, preparation of lysates using Triton X-100, preclearing, and immunoprecipitation using mAbs coupled to CNBr-activated Sepharose 4B (Pharmacia) were performed as described by Meuer *et al.* (1) unless otherwise specified. Immunoprecipitates were subjected to SDS/PAGE under reducing conditions. Standard molecular mass markers (New England Nuclear) of 90, 69, 46, 30, and 18 kDa were electrophoresed in parallel for molecular mass determination. For coprecipitation of Ti α/β structure using anti-CD3 mAbs, 5×10^7 cells were labeled using 1 mCi of ^{125}I (1 Ci = 37 GBq) before lysis for 45 min on ice with 10 mM triethanolamine (pH 7.6) containing 1% digitonin (21). Lysates were precleared several times with CNBr-activated Sepharose 4B beads coated with an irrelevant antibody before being incubated overnight at 4°C with beads coated with anti-mouse CD3ε (145-2C11) or anti-human CD3ε (Leu4) antibodies. Immunoprecipitates were washed extensively with lysis buffer before SDS/PAGE analysis.

IL-2 Production Assay. For all IL-2 production assays, 10^5 T cells were cultured in triplicates in a final volume of 200 μl of medium per well of 96-well round-bottom plates. For stimulation with antigen, 10^5 A20 presenting cells per well were cocultivated with the T cells and ovalbumin (1 mg/ml). For stimulation with mAbs coupled to beads (≈2 mg of mAb per ml of beads) ≈50 beads were added per well. For stimulation with plastic-bound mAbs, purified mAbs were incubated for 2 hr at room temperature at 100 μg/ml (found to be saturating for IL-2 production) in 50 μl of isotonic phosphate-buffered saline (pH 7.4). Plates were washed extensively with serum containing medium prior to the addition of the T cells. The supernatant from each assay was harvested after a 24-hr incubation. The IL-2 content was determined by incubating the IL-2-dependent cell line CTLL20 (at 10^4 cells per well) with 1:2 serial dilutions of the supernatant. The incorporation of [^{3}H]thymidine was determined by liquid scintillation counting after 18 hr. A titration curve with 1:2 serial dilutions of recombinant IL-2 (Biogen) was included in each experiment to quantify IL-2 secretion.

RESULTS AND DISCUSSION

Human CD3ε Subunit Can Be Stably Expressed at the Cell Surface of a Murine T-Cell Line. The cDNA coding for the human CD3ε subunit was introduced into the murine T–T hybridoma cell line 3DO54.8 that is specific for ovalbumin in the context of I-A^d class II MHC antigen (16) by using recombinant retrovirus infection or protoplast fusion. Transfected cells were selected for their resistance to G418 and analyzed for surface expression of the human CD3ε in an indirect immunofluorescence assay using the anti-human CD3ε mAb Vit3b. Five transfected cell lines, named ε1–ε5, reactive with mAb Vit3b, were selected for further analysis.

A representative human CD3ε$^+$ clone (clone ε2) reacted with each of the three anti-human CD3 mAbs used (i.e., Vit3b, Leu4, and RW2-8C8), but none of these mAbs bound to the parental line 3DO54.8 (Fig. 1*A*). In Western blot experiments using purified human CD3 complex, mAbs Vit3b and Leu4 have been shown (17) to react with the CD3ε subunit whereas no reactivity has been observed (17) with mAb RW2-8C8. These results suggest that mAb RW2-8C8 recognizes an epitope on the human CD3ε subunit that is more sensitive to denaturation than those recognized by mAbs Vit3b and Leu4. Further confirmation of cell surface expression of the human CD3ε subunit was obtained from immunoprecipitation studies using surface-labeled cells (Fig. 1*B*). Anti-human CD3 mAb Vit3b specifically immunoprecipitated a polypeptide from the ε2 cell line that comigrates with the CD3ε subunit from the human T-cell line Jurkat (detected as a 22-kDa species) (Fig. 1*B*). Thus, the human CD3ε subunit can be expressed in a stable way on the cell surface of a murine T-cell line and displays epitopes characteristic of its native configuration on human T cells. Note that mAb 2C11, specific for the murine CD3ε subunit (18), reacts similarly with the parental and the ε cell lines (Fig. 1), establishing the presence of the TCR on 3DO54.8 cells and showing that expression of the human CD3ε chain does not significantly affect expression of its murine counterpart.

Human CD3ε Subunit Is Expressed on Murine T Cells as a Part of a Functional TCR. In response to interaction with specific antigen and MHC or to crosslinking by anti-murine CD3 mAb, murine T–T hybridomas, such as 3DO54.8 cells, express the IL-2 gene and secrete IL-2. We, therefore, asked whether anti-human CD3ε could, in the same way, induce IL-2 secretion by binding to the ε cell lines. Human-CD3ε-positive clones ε1–ε5 as well as the parental line 3DO54.8 and cell line CD2 FL (derived by transfection with the CD2 cDNA) were assayed for IL-2 secretion after various types of stimulation. Although there was clonal variability in the response, all cell lines secreted high quantities of IL-2 when incubated with ovalbumin in the presence of I-A^d antigen-presenting cells (Fig. 2*A*). In addition, all cell lines produced IL-2 in response to the anti-murine CD3ε mAb with a pattern similar to that induced by ovalbumin plus I-A^d (Fig. 2*B*). However, IL-2 production induced by antibody 2C11 represented 30–100% of the production measured after antigen stimulation. In contrast, only cell lines expressing the human CD3ε subunit were induced to produce IL-2 upon stimulation

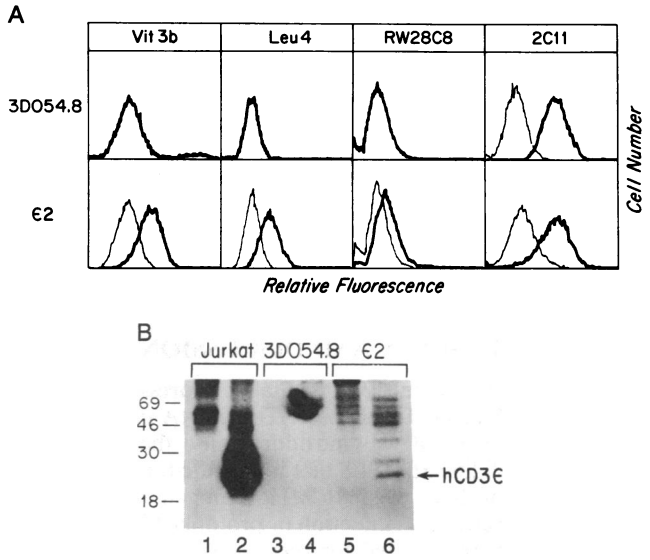

FIG. 1. Cell surface expression of human and murine CD3ε subunits in a murine T-T hybridoma cell line transfected with human CD3ε cDNA. (*A*) Flow cytometric analysis. Parental line 3DO54.8 and transfected line ε2 were incubated with anti-human CD3ε mAbs Vit3b, Leu4, or RW2-8C8 or with the anti-murine CD3ε mAb 2C11 (1:400 dilution of ascites fluid or purified mAb at 10 μg/ml). After subsequent incubation with a fluoresceinated goat anti-mouse antiserum [1:40 dilution (Meloy Laboratories)], the fluorescence intensity of 10,000 cells (thick line) was measured on an Epics V cell sorter. Nonspecific fluorescence was assessed using an irrelevant mAb for the first incubation step (thin line). Histograms represent the number of cells (ordinate) vs. $\log_{10}$ fluorescence intensity (abscissa). (*B*) Immunoprecipitation of human CD3ε (hCD3ε) from cell lysates. ^{125}I-surface-labeled cells were lysed using 1% Triton X-100. Lysates (equivalent to 4×10^6 cells) were immunoprecipitated with mAbs coupled to Sepharose beads and subjected to SDS/PAGE on a 12.5% polyacrylamide mini-gel under reducing conditions. A 48-hr autoradiograph of the immunoprecipitation with an irrelevant anti-CD4 mAb (lanes 1, 3, and 5) and anti-human CD3ε mAb Vit3b (lanes 2, 4, and 6) is shown. Molecular masses in kDa are shown.

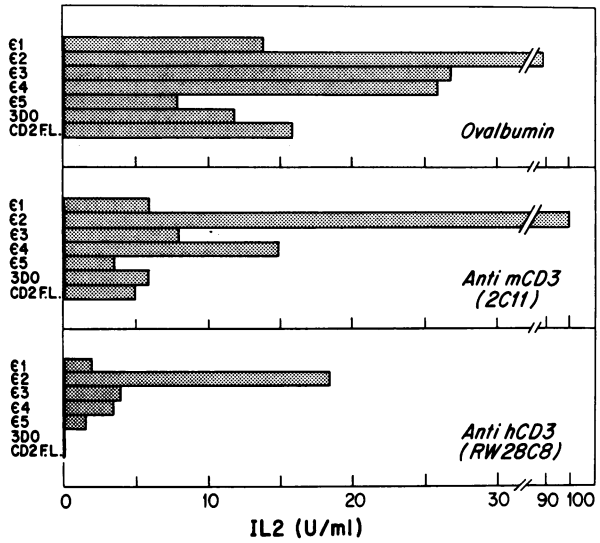

FIG. 2. IL-2 production of ε cell lines in response to antigen and crosslinked anti-murine or anti-human CD3ε mAbs. Clones ε1 and ε2 were derived from parental cell line 3DO54.8 (3DO) after transfection with the human CD3ε cDNA using retrovirus infection and clones ε3–ε5 were derived similarly using protoplast fusion. The control cell line CD2FL results from the transfection of 3DO54.8 cells with an irrelevant gene. Cells were stimulated with ovalbumin in the presence of antigen-presenting cells (*A*) or in anti-murine (m) CD3ε 2C11 (*B*) or anti-human (h) CD3ε RW2-8C8 (*C*) mAb-coated wells and IL-2 production was determined (limit, <0.25 μg/ml). U, unit(s).

with mAb RW2-8C8 (anti-human CD3ε) (Fig. 2*C*). The amount of IL-2 produced ranged from 18% to 50% of the amount secreted after mAb 2C11 stimulation. Other anti-human CD3ε mAbs (Vit3b and Leu4) induced significant IL-2 secretion from clone ε2 whereas stimulation of parental line 3DO54.8 did not produce detectable amounts of IL-2 (data not shown).

These results indicate that the human CD3ε subunit can target activation signals when expressed in a murine T-cell clone, strongly suggesting it is incorporated into a functional hybrid TCR. Further support for this hypothesis is provided by biochemical evidence for a noncovalent association between the introduced human CD3ε subunit and endogenous murine CD3 and Ti components (*infra vida*). In view of earlier studies documenting assembly of a physiologic TCR comprised of murine Ti α and β subunits with the CD3 subunits of the human Jurkat T-cell line, it would appear that murine and human TCR elements are sufficiently homologous at a structural level to allow functional interspecies complementation (14).

Deletion of the Cytoplasmic Domain of the Human CD3ε Subunit Does Not Prevent Assembly of a Functional TCR in Murine T Cells. After establishing a system whereby an isolated human CD3 subunit could be studied, we focused our attention on the cytoplasmic region of CD3ε. In particular, the high degree of amino acid homology in the CD3ε intracellular domains between the human and murine species (22) led us to investigate the role of this region in the assembly and/or function of the TCR. A mutant CD3ε cDNA was constructed by linker insertion of a termination codon on the carboxyl-terminal side of the sequence coding for the transmembrane domain. As a result, the mutant gene, termed εΔC, codes for a truncated form of CD3ε protein that lacks the last 49 of the 55 amino acids encoded in the cytoplasmic domain of the wild-type protein (Fig. 3*A*). The transfection of the murine εΔC gene into 3DO54.8 cells gave rise to cells reactive with mAbs Leu4 and Vit3b (termed cell lines εΔC1–εΔC5) to a similar extent as transfected cells that express wild-type CD3ε protein (Fig. 4*A* and data not shown).

To confirm that εΔC clones contained the mutant gene, Southern blot analysis was performed using genomic DNA from parental cell line 3DO54.8, one representative ε clone (ε4), and two representative εΔC clones (εΔC1 and εΔC2). As a result of the insertion of the mutagenic linker into the *Bam*HI fragment, which contains the CD3ε cDNA, the unique *Sty* I site present in the wild-type gene disappeared and an *Xba* I site was introduced close to that position (see Fig. 3*A*). With any of the three transfected clones, a 1400-bp *Bam*HI fragment was detected by the CD3ε cDNA probe whereas no signal was observed with the parental cell line at the hybridization stringency used (Fig. 3*B*, lanes 1–4). A fainter 730-bp band was also visible with ε2 DNA (lane 2), perhaps corresponding to a fragment of the CD3ε gene, which had been inserted as well. After subsequent digestion with *Sty* I, the 1400-bp *Bam*HI fragment derived from the DNA of the ε4 clone was cleaved into two distinct fragments of 600 and 800 bp while the DNA of the two εΔC clones was unaffected (Fig. 3*B*, compare lane 6 with lanes 7 and 8). As expected, the reciprocal pattern was observed when *Xba* I was used instead of *Sty* I (Fig. 3*B*, lanes 10–12).

To demonstrate directly that a truncated CD3ε chain was being produced in εΔC mutants, immunoprecipitation studies from lysates of ^{125}I-labeled clones were performed. As shown in Fig. 3*C* and consistent with results in ref. 17, the endogenous murine 21-kDa CD3 γ subunit and the CD3 δ/ε doublet detected as species of 26 and 25 kDa, respectively, were coimmunoprecipitated by anti-murine CD3ε mAb 2C11 from 3DO54.8 and εΔC2 cells (lanes 1 and 2). In contrast, immunoprecipitation using anti-human CD3ε Leu4 mAb specifi-

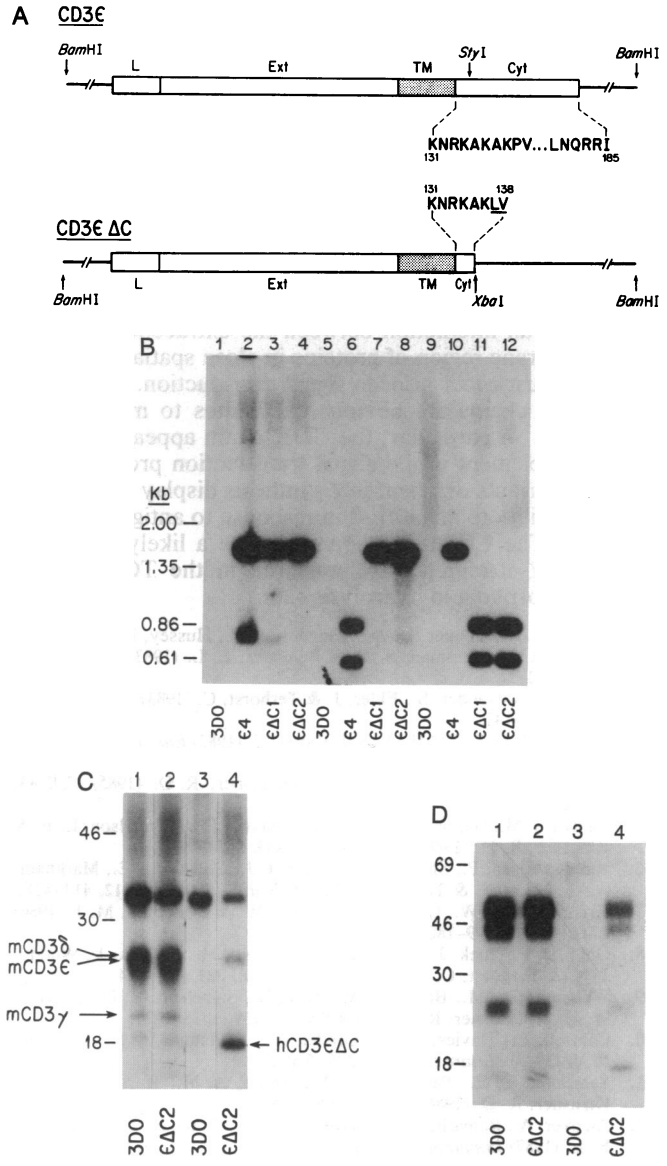

FIG. 3. Analysis of murine T-cell lines resulting from the transfection with a cDNA coding for a truncated human CD3ε. (*A*) Structure of the wild-type (CD3ε) and mutant (CD3εΔC) cDNAs and of the corresponding proteins. Noncoding and coding sequences are represented by thin lines and boxes, respectively. The first and last amino acids (using the single-letter amino acid code) of the cytoplasmic domains are shown. The last two amino acids found in the CD3εΔC protein are not encoded in the normal protein. L, leader; Ext, extracellular; TM, transmembrane; Cyt, cytoplasmic. (*B*) Southern analysis of the genomic DNA from CD3ε or CD3εΔC gene-transfected cell lines. DNAs were hybridized with a CD3ε DNA probe covering the entire cDNA insert. Lines ε4, εΔC1, and εΔC2 were obtained using the protoplast fusion method. Lanes: 1–4, *Bam*HI; 5–8, *Bam*HI and *Sty* I; 9–12, *Bam*HI and *Xba* I. Kb, kilobases. (*C*) Immunoprecipitation of murine (m) and truncated human (h) CD3ε from cell lysate. Immunoprecipitations were performed with murine (lanes 1 and 2) or human (lanes 3 and 4) anti-CD3ε mAbs coupled to protein A-Sepharose using lysates from 20×10^7 cells. Immunoprecipitates were analyzed on a 10% SDS/polyacrylamide gel under reducing conditions and the gel was autoradiographed for 7 days. (*D*) Coprecipitation of murine Ti α/β with anti-mouse (lanes 1 and 2) or anti-human (lanes 3 and 4) CD3ε mAbs. Cells were lysed in 10 mM triethanolamine (pH 7.6) containing 1% digitonin and immunoprecipitations were performed using mAb 2C11- or Leu4-coated beads. Molecular masses are in kDa.

cally identified two polypeptides of 17 and 26 kDa in the εΔC2 but not in the 3DO54.8 lysate (compare lanes 4 and 3). This

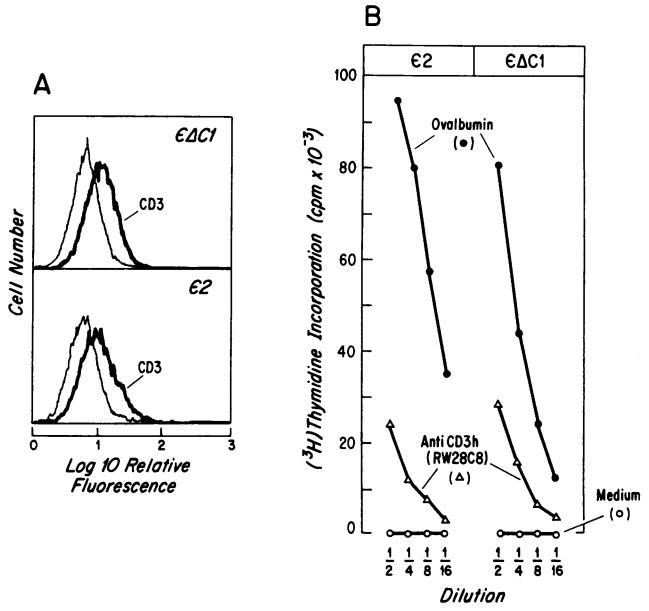

FIG. 4. Comparison of murine cell lines expressing the wild-type and truncated human CD3ε. (*A*) Cell surface expression of the human CD3ε subunit in ε2 and εΔC1 cells. Anti-human CD3ε mAb Leu4 was used to stain the cells in an indirect immunofluorescence assay (thick line). (*B*) IL-2 production of ε2 and εΔC1 cells in response to antigen or to anti-human CD3 mAbs. Stimulation was performed with either antigen- or anti-human (h) CD3ε mAb RW2-8C8-coated to plastic. Results are expressed as cpm of [^{3}H]thymidine incorporated by the IL-2-dependent CTLL20 cells at various dilutions of supernatants and represent the average of triplicate experiments.

result represents the coprecipitation of the truncated human CD3ε protein, expected to be ≈5 kDa shorter than the full-lengh 22-kDa protein (Fig. 1*C*), with the endogenous murine 26-kDa CD3δ subunit. The relatively low efficiency of labeling and/or coimmunoprecipitation of the murine CD3γ chain by mAb Leu4 (see lanes 1 and 2) might explain the absence of detectable murine CD3γ polypeptide in the εΔC2 precipitate. Note that the 35-kDa band present in all lanes represents a protein nonspecifically immunoprecipitated by protein A-Sepharose since it was identified in immunoprecipitates from the same lysate using anti-CD4 mAb coupled to protein A-Sepharose (data not shown). Consistent with this interpretation, the 35-kDa band was not observed when immunoprecipitates were obtained using mAbs bound to CNBr-activated Sepharose beads (Figs. 1*B* and 3*D*). Immunoprecipitations with anti-mouse or anti-human CD3ε under conditions that preserve the noncovalent association between CD3 chains and Ti α/β clearly established that both antibodies similarly coprecipitate the clonotype (resolving as two bands of 44 and 51 kDa, respectively) from the εΔC2 lysate. In contrast, only the anti-mouse CD3ε mAb was found to coprecipitate other TCR components from 3DO54.8 cells (Fig. 3*D*). This result establishes that a truncated CD3ε subunit associates with murine CD3–Ti components.

Since the cytoplasmic domain of the CD3ε subunit is not a requirement for its cell surface expression in the murine T–T hybridoma cell lines, it was possible to determine whether the εΔC cell lines could be activated in a fashion analogous to ε cell lines when stimulated with mAbs directed to the external domain of human CD3ε. As shown in Fig. 4*A*, cell lines ε2 and εΔC1, which express equivalent levels of surface CD3ε, were used for comparison. When incubated with anti-human CD3ε mAb RW2-8C8, both clones were induced to produce IL-2 (Fig. 4*B*) whereas no detectable IL-2 was produced by the parental cell line 3DO54.8 (data not shown). Although mAb Vit3b coupled to beads induced less IL-2 production than

mAb RW2-8C8, significant and comparable amounts of IL-2 were generated from clones εΔC1 and ε2 (data not shown). IL-2 production of clone εΔC1 could be directly compared to that of clone ε2 on a quantitative basis. In addition, other clones of the εΔC series were reproducibly found to produce IL-2 upon stimulation with mAb RW2-8C8 at a level similar to that of clones ε1 or ε4 (data not shown).

The ability of anti-human CD3ε mAb to trigger activation in the εΔC series of T cells might result from an association of several CD3ε subunits (i.e., one or more endogenous murine CD3ε subunits and a truncated human CD3ε subunit) with other TCR subunits in a given antigen–MHC receptor complex, thereby overcoming a potential functional deficit of the human CD3ε truncation. If this were the case, then there should be a linked surface coexpression of murine and human CD3ε subunits. To examine this possibility, the murine CD3ε subunit of clone εΔC2 was modulated from the surface by preincubation with mAb 2C11 for 18 hr at 37°C and, subsequently, surface expression of human CD3ε was examined. Although mAb 2C11 modulation removed ≥90% of murine CD3ε subunits, there was no decrease in human CD3ε expression after mAb 2C11 incubation as compared to modulation of εΔC2 cells with control antibody (data not shown).

Implications. In view of the general perception that the CD3 subunits are involved in signal transduction, apparent functional integrity of the truncated CD3ε subunit is surprising. A high degree of conservation in primary structure of protein domains across species is generally assumed to reflect preservation of a particular biologic function. One, therefore, would have anticipated this region to be important for the assembly of the TCR and/or the transduction of activation signals based upon the extensive homology observed in the cytoplasmic regions of the murine and human CD3ε (95% vs., for example, 47% in their external domain) (22). Nevertheless, neither function is significantly affected by the deletion of 49 of the 55 amino acids of the CD3ε cytoplasmic domain. The truncated CD3ε molecule is expressed at the cell surface and can target activation signals. The CD3ε cytoplasmic domain might, however, be required for functions that have not been addressed in the present study, such as endocytosis and the recyling process.

Presumably, the charged amino acids found in the transmembrane region of the individual TCR components (a negative and positive charge for the CD3 and Ti subunits, respectively) may play a role in the assembly process in addition to a proposed function in stabilizing the CD3–Ti complex (6–9). However, based on the present results, putative signals required for TCR assembly do not reside in the CD3ε cytoplasmic domain.

Since the present study was performed with a chimeric complementation system, the possibility that the endogenous murine CD3ε cytoplasmic domain might be involved in the transmembrane signaling upon stimulation through the human subunit has to be considered. If this were the case, then either a given functional CD3 complex must contain more than one CD3ε subunit or the physiologic TCR must consist of several clustered antigen-binding CD3–Ti complexes, one or more of which contains an intact murine CD3ε subunit. The former possibility is not excluded but unlikely for several reasons. (*i*) Studies of CD3ε–Ti complex structure have led to the conclusion that the stochiometry of Ti α/Ti β/CD3γ/CD3δ/CD3ε is 1:1:1:1:1 (23). (*ii*) Consistent with this view is the lack of detectable human CD3εΔC observed in anti-murine CD3ε precipitates of εΔC2 (see Figs. 3*C*, lane 2, and 4*D*, lane 2). Furthermore, modulation of the TCR complexes containing murine CD3ε by mAb 2C11 does not alter mAb Leu4 reactivity. Thus our results are consistent with the view that coupling of the TCR to phospholipase C resulting in the generation of second messengers such as inositol phosphates and Ca^{2+} probably does not operate through an association of a transducing element (i.e., a guanine nucleotide–binding regulatory protein) with the CD3ε cytoplasmic domain.

We determined that the majority of murine anti-human CD3 mAbs (14/17 mAbs) recognize the CD3ε subunit (24). In addition, the hamster anti-mouse CD3 mAb is directed against CD3ε (18). This set of observations implies that the external segment of CD3ε in both species is readily expressed on the surface of T lymphocytes. Whether the CD3ε extracellular segment is contacted by Ti subunits or other proteins after antigen–MHC interaction is a matter of speculation. However, since binding of mAbs to the external portion of CD3ε chains lacking their cytoplasmic domain leads to T-cell activation, an interaction between the extracellular and/or transmembrane region of proteins in close spatial proximity to CD3ε must occur prior to signal transduction. Any of the other CD3 chains are obvious candidates to mediate this interaction. In particular, the CD3ζ chain appears to be an important element in the signal transduction process given that cell variants deficient in ζ synthesis display a markedly reduced ability to make IL-2 in response to antigen stimulation (25). The CD3η also appears to be a likely candidate since CD3ζ–η mutants are impaired in the TCR-coupled inositol phospholipid hydrolysis (26).

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