

INDEPENDENT ASSOCIATION OF T CELL RECEPTOR
 β AND γ CHAINS WITH CD3 IN THE SAME CELL

BY FRITS KONING,* W. LEE MALOY,* DAVID COHEN,† AND
JOHN E. COLIGAN*

*From the *Biological Resources Branch, National Institute of Allergy and Infectious Diseases; and the †Laboratory of Chemical Biology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892*

The antigen receptor (TCR) on effector T lymphocytes has been characterized as an $\alpha\beta$ chain heterodimer that is associated with the CD3 (T3) complex on the cell surface (1). Recently, a second CD3-associated heterodimer, composed of a γ gene product and a putative δ gene product, has been identified on a subset of human lymphocytes (2, 3) human thymocytes (4), and murine thymocytes (5). Because β and γ chain mRNA, in contrast to α chain mRNA transcripts, are abundant in immature murine thymocytes (6, 7) it was hypothesized that such thymocytes could express $\beta\gamma$ receptors that are replaced by $\alpha\beta$ receptors after translation of α chain mRNA (6, 7). However, no evidence exists that β and γ protein can be synthesized by a single cell. In this report we show that the human T cell line PEER, which expresses a CD3 associated TCR γ chain on the cell surface (8, 9), synthesizes a 38 kD β chain product that associates intracellularly with CD3. However, this CD3-TCR β complex is not found on the cell surface, presumably because no α chain protein can be synthesized (8). We conclude that β and γ proteins can be produced within the same cell and that they can independently form CD3-TCR β and CD3-TCR γ chain complexes. However, we could find no evidence for CD3-TCR $\beta\gamma$ complexes in this cell line.

Materials and Methods

Radiolabeling and Immunoprecipitation. Cells were surface labeled with ^{125}I as described previously (10). For biosynthetic labeling, $5\text{--}10 \times 10^7$ cells were washed twice with PBS/1% FCS and subsequently resuspended in 25–50 ml Met/Cys-free RPMI 1640 (Flow Laboratories, McLean, VA) supplemented with 20 mM Hepes, 5% FCS, and 0.5 mCi each of [^{35}S]Met and [^{35}S]Cys. After a 5-h labeling period, the cells were harvested and lysed in lysis buffer containing 0.5% NP-40 or 1% digitonin as described previously (10). After 15 min at 0°C, the lysates were spun for 15 min at 13,000 g. Supernatants were precleared for 16 h at 4°C with 50 μl normal rabbit serum and 100 μl protein A-agarose beads (PAA beads) (BRL, Bethesda, MD). After removal of the PAA beads by centrifugation, specific precipitations were carried out by adding 5 μl of antiserum to 50–100 μl of lysate. After 60 min, 5 μl of PAA beads were added for a second 60-min incubation. Subsequently the beads were washed four or five times with lysis buffer. Immunoprecipitates were analyzed on 12% SDS-PAGE gels under reducing conditions with subsequent autoradiography.

Antisera. Antisera specific for TCR β chains (anti- β), TCR γ chains (anti- γ), and CD3 δ

F. Koning is a recipient of a NATO Science Fellowship from the Netherlands Organization for the Advancement of Pure Research.

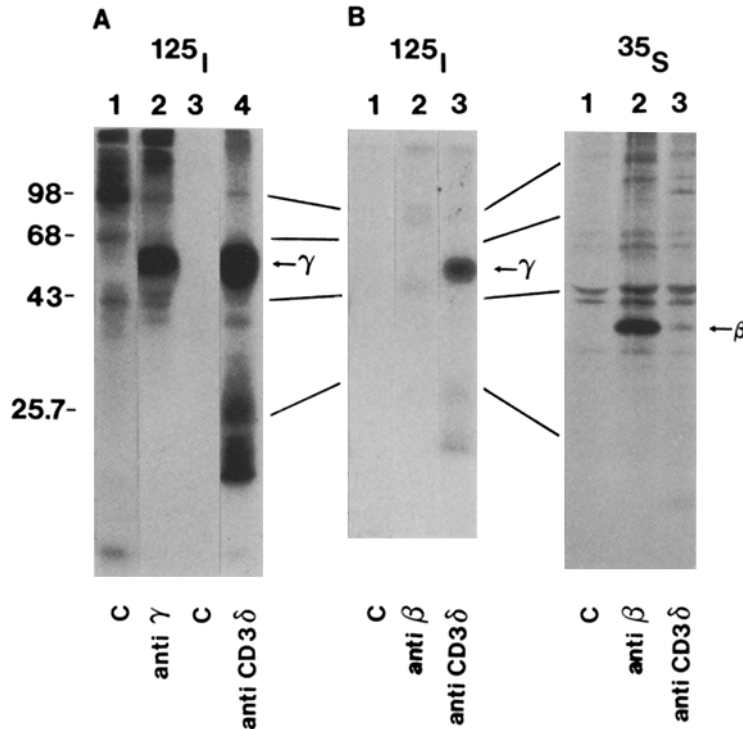


FIGURE 1. Analysis of CD3-associated molecules and TCR β chain expression on surface iodinated or biosynthetically labeled PEER cells. *A*, Cells were surface iodinated and lysed in either NP-40 (lanes 1 and 2) or digitonin lysis buffer (lanes 3 and 4). Precipitations were carried out with normal rabbit serum (lanes 1 and 3), anti-TCR γ chain antiserum (lane 2) and anti-CD3 δ antiserum (lane 4). *B*, Cells were cell-surface iodinated (^{125}I) or biosynthetically labeled (^{35}S), lysed in 1% digitonin buffer, and immunoprecipitations were carried out with normal rabbit serum (C, lanes 1), anti- β serum (lanes 2) and anti-CD3 δ serum (lanes 3).

(anti-CD3 δ) chains were raised by immunization of rabbits with specific COOH-terminal peptides as described previously (10).

Radiolabeling and Purification of Proteins for Subsequent NH_2 -Terminal Amino Acid Sequence Analysis. Proteins for amino acid sequence analysis were isolated from 2×10^8 PEER cells biosynthetically labeled with 1 mCi [^{35}S]Met and 5 mCi [^3H]Leu in 50 ml Met/Leu-free RPMI 1640 (Gibco, Grand Island, NY) supplemented with 5% FCS and 20 mM Hepes for 5 h. After washing twice in RPMI 1640, the cells were lysed in digitonin lysis buffer for 30 min at 0°C . After centrifugation at 13,000 g for 15 min, the lysate was precleared and immunoprecipitations were carried out using anti-CD3 δ and anti- β antiserum. The samples were applied to preparative 12% SDS-PAGE gels. After autoradiography, gel slices containing the proteins of interest were excised and the proteins were recovered by electroelution in 5 mM Tris-HCl, 0.1% SDS, pH 7.5, followed by ethanol precipitation (in the presence of cytochrome C as carrier protein). Protein pellets were dissolved in 50% acetic acid and subjected to sequence analysis on a Beckman Model 890M protein sequencer.

Results and Discussion

Although PEER expresses CD3 in association with a 55–60 kD TCR γ chain molecule on the cell surface (Fig. 1 *A*) (occasionally a 40 kD protein coprecipitates in anti-CD3 and anti-TCR γ chain precipitates which may represent the δ chain [9]), this cell line is also known to contain an mRNA transcript for the C β 2

constant region of the TCR (8, 11). To investigate if TCR β chain protein was expressed in the PEER cell line, PEER cells were either surface labeled with ^{125}I or biosynthetically labeled with [^{35}S]Met/Cys and subsequently lysed in digitonin lysis buffer (which has been demonstrated not to interfere with the association of CD3 and the TCR complex). Immunoprecipitations were carried out using normal rabbit serum (C), an anti-TCR β chain antiserum (anti- β) and an anti-CD3 δ antiserum (anti-CD3 δ) (10). The anti-CD3 δ antiserum precipitated CD3 in association with the 55–60 kD TCR γ chain from ^{125}I -labeled cells (Fig. 1B), whereas it precipitated CD3 in association with a 38 kD molecule from ^{35}S -labeled cells (Fig. 1B). The anti- β antiserum, which failed to specifically react with any surface molecules from ^{125}I -labeled cells (Fig. 1B), also detected a 38 kD molecule in the ^{35}S -labeled cells (Fig. 1B). The reactivity of both antipeptide antisera could be inhibited by the addition of the relevant peptides (not shown).

It was next of interest to determine whether the 38 kD protein that associated with CD3 intracellularly was a precursor to the γ chain associated with CD3 on the cell surface, or whether it was the 38 kD protein detectable intracellularly with the anti- β reagent. *N*-glycanase treatment (Genzyme, Boston, MA) of an anti- β chain and an anti-CD3 δ immunoprecipitate reduced the 38 kD proteins in each to 32 kD (not shown). Furthermore, preclearing of a ^{35}S -labeled lysate of PEER cells with the anti- β antiserum completely removed coprecipitation of the 38 kD band in the anti-CD3 δ precipitate (Fig. 2). In contrast, preclearing with the anti-CD3 δ antiserum only partially removed anti- β reactivity (Fig. 2), indicating that the majority of the β chain protein exists free of the CD3 complex. These results indicated that the 38 kD protein associated with CD3 intracellularly was the β chain. Definitive proof that the CD3-associated molecule is the TCR β chain was obtained by amino acid sequence analysis. The 38 kD bands precipitated with the anti- β and coprecipitated with the anti-CD3 δ antisera were isolated from cell lysates labeled with [^3H]Leu and [^{35}S]Met. Both proteins were found to have Leu at positions 9, 19, and 25, and Met at positions 11, 13, and 30 (Fig. 3). This sequence (with the exception of Leu at position 25) matches that described for TCR V β 7.1 (12), and indicates that in PEER V β 7.1 rearranged to C β 2 (11).

These results clearly show that the intracellular CD3-associated 38 kD molecule is a TCR β chain. Moreover, analysis of TCR chain mRNAs demonstrated that PEER contains a full-length transcript (1.3 kb) for TCR β chain, and confirmed previous results that this cell line expressed no TCR α chain mRNA but contains abundant, full-length TCR γ chain mRNA (not shown). Therefore, these results indicate that in PEER free β chain associates with the CD3 complex intracellularly in the absence of α chain, and suggest that the CD3-TCR β chain complex is not transported to the cell surface due to the absence of α chain protein. These results support previous suggestions that immature T cell lines lack TCR expression due to the absence of α chain protein (13).

After biosynthetic labeling, we were unable to detect CD3 in association with the TCR γ chain (or its precursor) even though free intracellular CD3 is available. This free intracellular CD3 is evident after preclearing with the anti- β antiserum (Fig. 2). This indicates that only a minor fraction (presently undetectable) of the intracellular CD3 complex associates with the TCR γ chain (or its precursor).

During murine thymic ontogeny, the γ and β genes are found to be rearranged

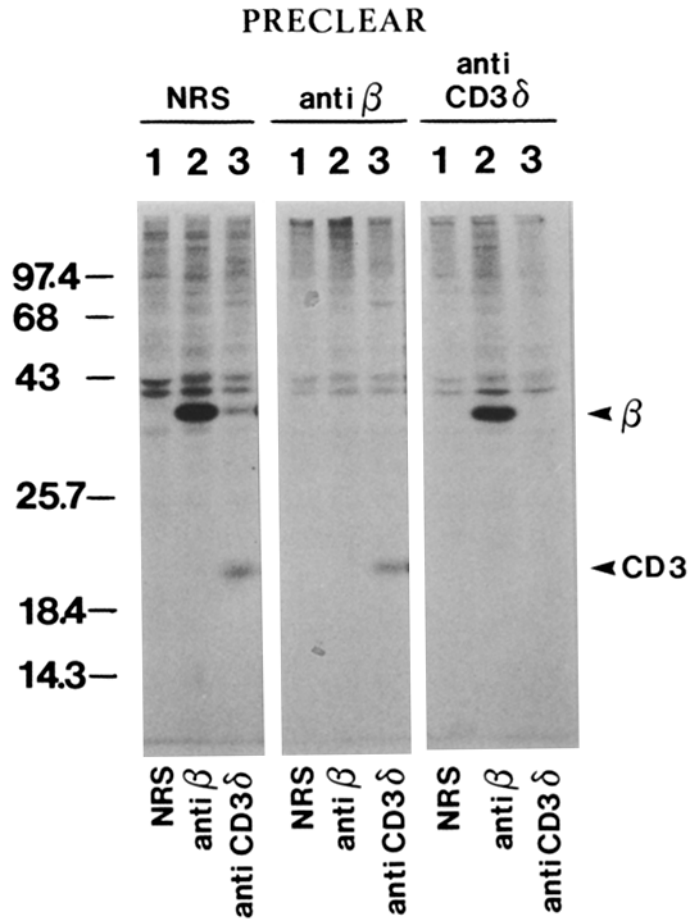


FIGURE 2. Sequential immunoprecipitations from ^{35}S -labeled lysates of PEER cells. PEER cells were metabolically labeled with $[^{35}\text{S}]\text{Met}$ and $[^{35}\text{S}]\text{Cys}$ and lysed in digitonin lysis buffer. After preclearing with either normal rabbit serum, anti- β or anti-CD3 δ , immunoprecipitations were carried out in these precleared samples with normal rabbit serum (lanes 1), anti- β (lanes 2) and anti-CD3 δ (lanes 3).

and transcribed several days before the α gene (6). This observation has led to the speculation that initially the β and γ gene products can associate to form a $\beta\gamma$ TCR that, after α gene rearrangement and transcription, is displaced by an $\alpha\beta$ TCR (6, 7). However, our observation that, in PEER, CD3-TCR β and CD3-TCR γ complexes are formed independently, without any evidence for a $\beta\gamma$ TCR, argues against formation of such a $\beta\gamma$ TCR. This would be compatible with the notion that the TCR γ and β chain bind to the same CD3 chain. Both human and murine TCR β and γ chains have a conserved positively charged lysine residue in their transmembrane region (14), which may play a role in stabilizing the CD3-TCR complex through formation of a salt bridge with the negatively charged amino acids found in the transmembrane regions of the CD3 subunits (14). Because the primary association between TCR ($\alpha\beta$) and CD3 (γ , δ , and ϵ) appears to be with TCR β -CD3 γ (15), the TCR β and γ chains might compete for binding to CD3 γ . At present it is not clear whether thymocytes

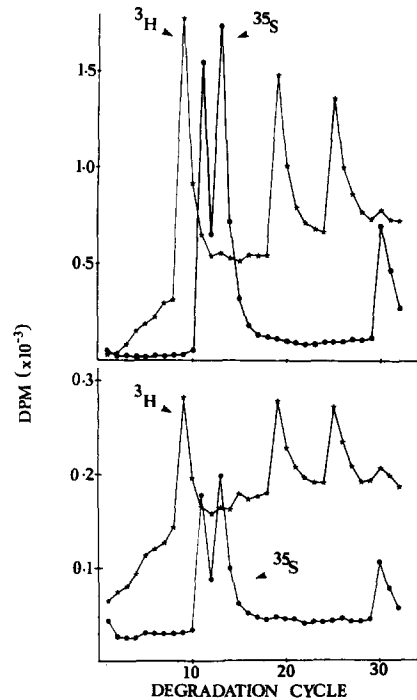


FIGURE 3. Partial NH₂-terminal amino acid sequence of [³H]Leu and [³⁵S]Met-labeled PEER β chain and CD3-associated 38 kD molecule. PEER cells were metabolically labeled with [³⁵S]-Met and [³H]Leu. From these cells, the 38 kD TCR β chain and the 38 kD CD3-associated proteins were isolated, and a partial NH₂-terminal amino acid sequence was determined. *Top*: 38 kD TCR β chain. *Bottom*: CD3-associated 38 kD protein.

expressing $\gamma\delta$ TCR form a separate lineage or that (part of) these thymocytes switch to $\alpha\beta$ TCR during maturation. In the latter case, the PEER cell line might represent a transitional stage.

Summary

We have demonstrated that the PEER cell line, which expresses a CD3-associated TCR γ chain on the cell surface, synthesizes TCR β chain intracellularly. A percentage of this TCR β chain associates with the CD3 complex intracellularly. These results indicate that TCR β and γ chains can be synthesized by one cell line, and that these chains can independently associate with the CD3 complex. However, the results argue against the formation of TCR $\beta\gamma$ chain complexes in this cell line.

We thank R. Sekaly for providing us with the PEER cell line, A. Lew and D. Pardoll for helpful discussions, R. Valas and M. Raum for technical assistance, R. Sekaly, E. O. Long and T. J. Kindt for critically reading the manuscript, and G. Shaw for typing the manuscript.

Received for publication 16 March 1987 and in revised form 11 May 1987.

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