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Immunology

Structure of the T-cell antigen receptor: Evidence for two CD3 ε subunits in the T-cell receptor–CD3 complex

(transfection)

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ABSTRACT **The T-cell antigen receptor (TCR) consists of heterodimeric glycoproteins (TCR $\alpha\beta$ or $\gamma\delta$) that demonstrate homology with immunoglobulins. Noncovalently associated with the $\alpha\beta$ (or $\gamma\delta$) heterodimer are at least five nonvariant proteins (CD3-γ, -δ, -ε, -ζ, and -η), which together comprise the TCR–CD3 complex. The stoichiometry of the antigen receptor has been assumed to be either $\alpha\beta\gamma\delta\varepsilon\zeta\zeta$ or $\alpha\beta\gamma\delta\varepsilon\zeta\eta$. In this paper we provide several lines of evidence that support the notion that the mature TCR–CD3 complex on the cell surface contains two CD3-ε polypeptide chains. Transfection of two murine T cell–T cell hybridomas with the human DNA encoding CD3-ε protein demonstrated that both murine and human CD3-ε chains were present within the same TCR–CD3 complex. Analysis of thymocytes isolated from transgenic mice that expressed high copy numbers of the human CD3-ε gene showed that the heterologous human CD3-ε subunits were coexpressed with murine CD3-ε in the same TCR–CD3 complex. Since CD3-ε was shown to form disulfide-linked homodimers both in human and murine T cells, the two CD3-ε subunits present in the TCR–CD3 complex were in direct contact with one another. The presence of two CD3-ε polypeptide chains in close proximity to one another in the TCR–CD3 complex may have important implications for its assembly and its signal transduction mechanisms.**

The T-cell antigen receptor (TCR) on thymus-derived lymphocytes (T cells), which recognizes antigen in association with either class I or class II major histocompatibility complex (MHC) products, consists of a heterodimer of glycoproteins, the α and β chain (1). A second form of the TCR, the $\gamma\delta$ receptor, also exists on a smaller fraction of peripheral T cells (2). The TCR heterodimers are noncovalently associated with at least five nonvariant polypeptides: CD3-γ, -δ, -ε, -ζ, and -η (3, 4). CD3-ζ exists either as a disulfide-linked homodimer or, in the minority of TCR–CD3 complexes, as a disulfide-linked heterodimer with the CD3-η chain (4). The CD3 proteins appear to play an important role in transport of the TCR heterodimer to the cell surface and signal transduction after the TCR binds its appropriate ligand.

The specific number of polypeptide chains that compose the TCR–CD3 complex on the cell surface has not been precisely determined. The stoichiometry has generally been assumed to be either $\alpha\beta\gamma\delta\varepsilon\zeta\zeta$ or $\alpha\beta\gamma\delta\varepsilon\zeta\eta$ (3, 4). In this paper, we analyze the stoichiometry of CD3-ε in the complex by expression of human CD3-ε (CD3-ε^h) in murine T cells. We provide evidence that there are two CD3-ε polypeptide chains in close proximity to one another within the mature TCR–CD3 complex expressed on the cell surface.

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MATERIAL AND METHODS

Cell Lines. The cell lines used as recipients for transfection were BY155.16, a murine T cell–T cell (T–T) hybridoma which was obtained by fusing BW5147 mouse lymphoma cells with T cells from a C57BL/6 mouse primed with human JY cells (5), and 3DO54.8, a murine T–T hybridoma that is I-A^d class II MHC antigen-restricted and ovalbumin specific (ref. 6; 3DO54.8 was a gift of Phillipa Marrack, National Jewish Center, Denver). SL12.4$\alpha\beta$ is a cell line produced by transfection of SL12.4 with the TCR α and β mRNAs from an arsonate/I-A^d-specific T-cell clone, AR-5 (7). HPB-ALL is a human leukemic T-cell line.

Transgenic Mice. Transgenic mice expressing the CD3-ε^h gene were made as described (8).

Antibodies. The monoclonal antibody (mAb), SP34, which is a murine IgG3 mAb and recognizes the CD3-ε^h protein, was obtained by immunization of BALB/c mice with purified human CD3 proteins (9). The anti-murine CD3-ε (CD3-ε^m) mAb 145-2C11 was a gift from Jeffrey Bluestone (University of Chicago) (10). HMT 3.2 antibody was obtained by immunization of Armenian hamsters with purified human CD3 proteins and was donated by Ralph Kubo (National Jewish Center, Denver). HMT 3.2 recognizes the murine CD3-γ and -δ subunits (unpublished data; B. Alarcon and C.T.). Leu-4 is a murine IgG1 mAb that recognizes the CD3-ε^h chain (11, 12).

Expression Vectors. The pSRα-Neo-CD3-ε^h expression vector was obtained in the following manner. A 1600-base-pair (bp) *Xho* I–*Xho* I fragment of the pCD-ε plasmid (13) containing the complete CD3-ε^h open reading frame was cloned into the unique *Xho* I site of the pSRα-Neo expression vector (provided by Ken Ichi Arai, DNAX; ref. 14). The pSRα-Neo vector utilizes neomycin (geneticin sulfate) resistance as a selectable marker.

DNA Transfer into T Cells. Transfection was accomplished by utilizing electroporation as described (15).

Immunofluorescence. Surface expression of antigens was detected by indirect immunofluorescence as described (7, 15) by using either normal mouse serum as a control or a 1:400 dilution of BALB/c ascites containing the Leu-4 mAb and 1 μg of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse F(ab')$_2$ antibody specific for IgG and IgM heavy and light chains (Tago). Two-color immunofluorescence was performed with the 145-2C11 mAb and a FITC-conjugated goat

Abbreviations: TCR, T-cell receptor; mAb, monoclonal antibody; T–T hybridoma, T cell–T cell hybridoma; MHC, major histocompatibility complex; CD3-ε^h and CD3-ε^m, human and mouse CD3-ε subunits, respectively.

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anti-hamster antibody specific for IgG and IgM heavy and light chains (Southern Biotechnology Associates, Birmingham, AL) followed by phycoerythrin-conjugated Leu-4 (Becton Dickinson). Normal mouse immunoglobulin directly conjugated with phycoerythrin (MsIgG-RD1, Coulter) was used as a control for nonspecific phycoerythrin immunofluorescence.

Radiolabeling. Cell surface iodination of lymphocytes with carrier-free $Na^{125}I$ (DuPont/NEN) was performed by the lactoperoxidase-catalyzed method as described (15).

Immunoprecipitation and Electrophoresis. Radiolabeled T cells were lysed on ice with an immunoprecipitation buffer containing 20 mM Tris·HCl, 0.15 M sodium chloride, 10 mM iodoacetamide, 1 mM phenylmethylsulphonyl fluoride, and 1 mg each of leupeptin, pepstatin, antipain, and chymostatin (small peptidase inhibitors) per ml at pH 7.6 with 1.0% digitonin with or without 0.12% Triton X-100 as detergents on ice. Immunoprecipitates were prepared from the lysates as described (15) and resuspended in 30 μl of Laemmli sample buffer without reducing agents (16). The immunoprecipitates were analyzed by two-dimensional nonreducing/reducing polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) as described (17).

Immunoblotting. Leu-4 immunoprecipitates, separated by two dimensional nonreducing/reducing SDS/PAGE, were analyzed by Western blotting with the SP34 mAb as described (18).

RESULTS

Stable Expression of the CD3-ε^h Subunit on the Cell Surface of Two Murine T-Cell Hybridomas. The CD3-ε^h subunit was expressed in an HLA-DR-specific murine T–T hybridoma, BY155.16, and also in an I-A^d class II MHC antigen-restricted ovalbumin-specific murine T–T hybridoma, 3DO54.8, by transfection with the pSRα-Neo-CD3-ε^h expression vector. After DNA transfer, neomycin-resistant cells were selected and analyzed by indirect immunofluorescence by using the CD3-ε^h-specific mAb, Leu-4 (Fig. 1). All of the neomycin-resistant transfectant cell lines analyzed (one of which is shown here for each recipient murine T-cell line) were positive for staining with the Leu-4 mAb, while transfectants made with the pSRα-Neo vector alone were negative. The proportion of transfectant cells that expressed the CD3-ε^h protein on their cell surface ranged from 40% to 95%.

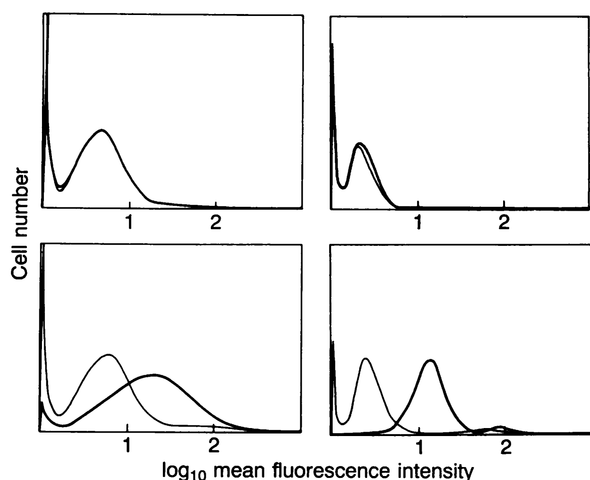

FIG. 1. Immunofluorescence of BY155.16 and 3DO54.8 transfectants. BY155.16 (*Upper Left*) and 3DO54.8 (*Upper Right*) and their transfectants (*Lower Left* and *Lower Right*, respectively) were stained with the Leu-4 mAb as described (heavy line). Nonspecific immunofluorescence is indicated by the thin line.

Two-color immunofluorescence was performed to determine whether the CD3-ε^h subunit was present on the same cell as the CD3-ε^m subunit. At least 60% of the transfected cells concomitantly expressed the CD3-ε^h and CD3-ε^m subunits (Fig. 2). These results indicated that the CD3-ε^h subunit was expressed on the surface of a murine T-cell line in conjunction with the CD3-ε^m chain.

Both CD3-ε^h and CD3-ε^m Subunits Are Expressed Within the Same TCR–CD3 Complex. After demonstrating that both CD3-ε^h and CD3-ε^m could be coexpressed on the surface of the same T cell, it was important to determine whether both were present in the same TCR–CD3 complex. To do this, surface ^{125}I-labeled protein was immunoprecipitated with subunit-specific mAbs. When the lysates were immunoprecipitated with the CD3-ε^h-specific mAb (SP34) and analyzed by two-dimensional nonreducing/reducing SDS/PAGE, both CD3-ε^h and CD3-ε^m subunits could be observed in isolates from the transfected BY155.16 and 3DO54.8 cell lines (Fig. 3*A Lower*). Similarly, when labeled protein from the BY155.16 cell line was analyzed with the CD3-ε^m-specific mAb (145-2C11) and an independent mAb that recognized the murine CD3-γ- and -δ-specific subunits (HMT 3.2), both CD3-ε^m and CD3-ε^h subunits were coisolated in the transfectant (Fig. 3*B Lower*). No components of the TCR–CD3 complex were isolated from the untransfected BY155.16 and 3DO54.8 cell lines with the SP34 mAb (Fig. 3*A Upper*), and only the CD3-ε^m chain was detected with the 145-2C11 and HMT 3.2 mAbs (Fig. 3*B Upper*). Therefore, it appeared that the TCR–CD3 complex on the surface of the BY55.16 and 3DO54.8 transfectants contained both CD3-ε^h and CD3-ε^m subunits in addition to CD3-γ, -δ, and -ζ and the TCR $\alpha\beta$ heterodimer (indicated in the figures).

To confirm that the TCR–CD3 complex on the surface of the transfectants contained both the CD3-ε^h and CD3-ε^m

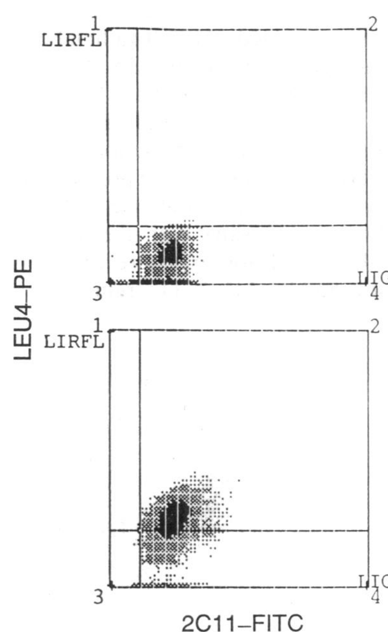

FIG. 2. Two-color immunofluorescence of 3DO54.8 (*Upper*) and a transfectant of 3DO54.8 (*Lower*). Both were stained with the 145-2C11 mAb and a fluorescein isothiocyanate (FITC)-conjugated goat anti-hamster F(ab′)$_2$ antibody (2C11-FITC; ordinate) followed by Leu-4 directly conjugated with phycoerythrin (Leu4-PE; abscissa) as described. Distribution in quadrants for 3DO54.8 are 1, 0.0%; 2, 0.24%; 3, 6.76%; and 4, 93.01% and for its transfectant are 1, 0.33%; 2, 60.42%; 3, 1.83%; and 4, 37.42%. Abscissa: log integrated green fluorescence. Ordinate: log integrated red fluorescence.

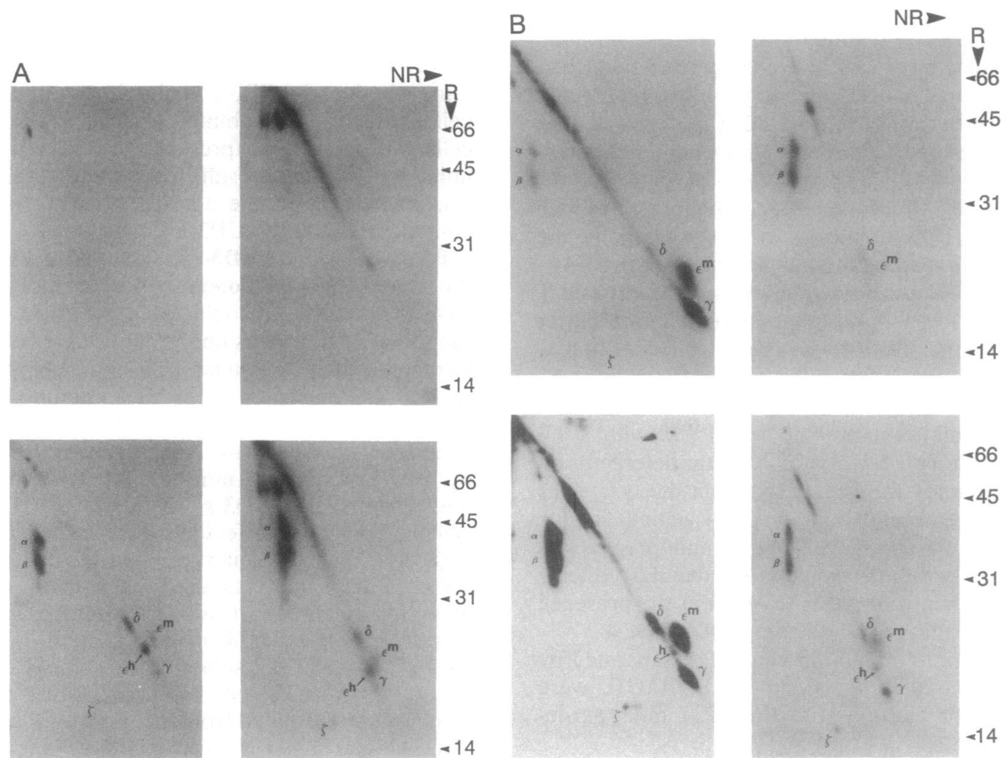

FIG. 3. Immunoprecipitation of CD3-ϵ^h from transfectants of BY155.16 and 3DO54.8 cells. Untransfected (*Upper*) and transfected (*Lower*) BY155.16 cells (*A Left* and *B*) and 3DO54.8 cells (*A Right*) were radiolabeled with $Na^{125}I$ by the lactoperoxidase-catalyzed method, lysed with immunoprecipitation buffer containing 1% digitonin with (BY155.16 cells) or without (3DO54.8 cells) 0.12% Triton X-100, and immunoprecipitated with the SP34 mAb (*A*), 145-2C11 mAb (*B Left*), or HMT 3.2 mAb (*B Right*). The immunoprecipitates were analyzed on a SDS/12.5% polyacrylamide gel under two-dimensional nonreducing (NR)/reducing (R) conditions. The location of CD3-ϵ^h (ϵ^h) and CD3-ϵ^m (ϵ^m) is indicated. The molecular mass markers in kDa are shown on the right.

subunits and to determine what fraction of the CD3-ϵ^h complexed with the CD3-ϵ^m subunit, an immunodepletion experiment was performed. An ^{125}I-radiolabeled protein lysate from a transfectant of the BY155.16 cell line was first immunoprecipitated with either the CD3-ϵ^m-specific mAb (145-2C11) or the CD3-ϵ^h-specific mAb (SP34). Subsequently, the supernatants were immunoprecipitated with the alternative antibody, and the isolated protein was resolved by two-dimensional nonreducing/reducing SDS/PAGE (Fig. 4). Fig. 4 *Left* shows the results with the nondepleted lysate using the SP34 mAb. Prior exposure of the lysate to the CD3-ϵ^m-specific mAb, 145-2C11, removed most of the CD3-ϵ^h subunit (Fig. 4 *Center*). However, there did appear to be some residual CD3-ϵ protein after immunodepletion with the 145-2C11 antibody (arrow). Conversely, after treatment with the CD3-ϵ^h-specific mAb, significant amounts of a normal mouse complex were isolated that only contained the CD3-ϵ^m subunit (Fig. 4 *Right*). These results suggested, therefore, that three types of receptors existed on the cell surface of the transfectants that contained either the CD3-ϵ^m or CD3-ϵ^h subunit, or both.

CD3-ϵ^h and CD3-ϵ^m Subunits Are Coexpressed Within the Same TCR–CD3 Complex on the Cell Surface of Thymocytes from Transgenic Mice. We have recently described a trans-

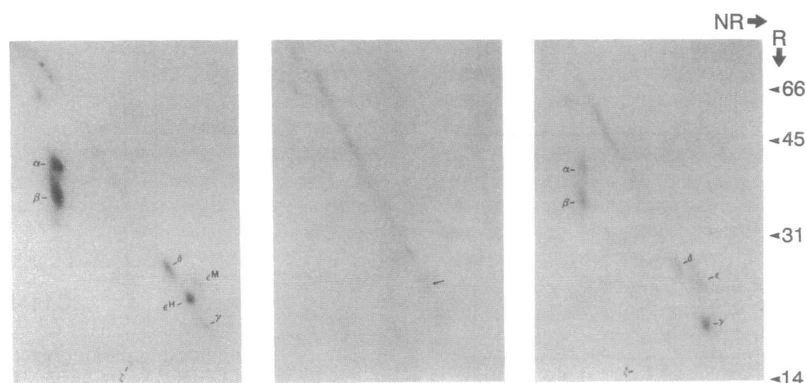

FIG. 4. Immunodepletion of the CD3-ϵ^h subunit from a BY155.16 transfectant. A lysate from radioiodinated material was prepared from the transfectant of BY155.16 by using an immunoprecipitation buffer containing 1% digitonin and 0.12% Triton X-100. The lysate was either immunoprecipitated directly with the SP34 (*Left*), or immunodepleted with the 145-2C11 mAb followed by immunoprecipitation with SP34 (*Center*), or immunodepleted with the SP34 mAb followed by immunoprecipitation with 145-2C11 (*Right*). The final immunoprecipitates were analyzed by two-dimensional nonreducing (NR)/reducing (R) SDS/PAGE on a 12.5% gel. The arrow in *Center* indicates the position of CD3-ϵ^h. The molecular mass markers in kDa are shown on the right.

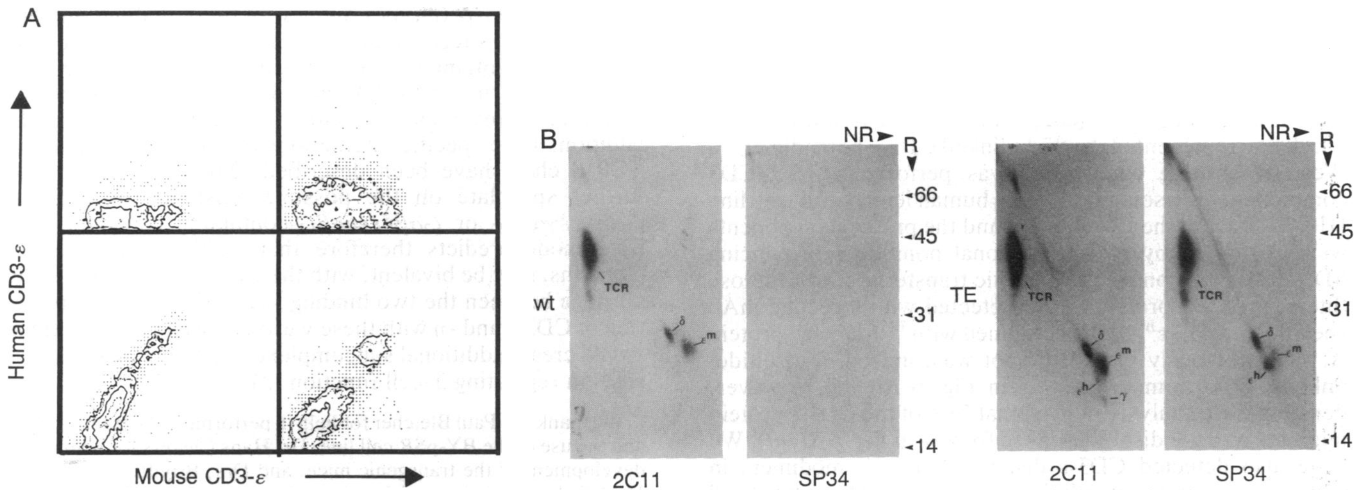

FIG. 5. Expression of the CD3-ε^h protein in 1-month-old hemizygous transgenic mice. (*A*) Two-color immunofluorescence of wild-type (*Upper*) and transgenic (*Lower*) mouse thymocytes from thymus (*Left*) and spleen (*Right*). The ordinate represents green fluorescence (145-2C11 mAb), and the abscissa represents red fluorescence (Leu-4 mAb). (*B*) Immunoprecipitation of the CD3-ε^h protein from a transgenic mouse. Lysates of ^{125}I-radiolabeled material were prepared from thymocytes of a wild-type mouse (WT) and transgenic mouse (TE) by using an immunoprecipitation buffer that contained 1% digitonin. The lysates were immunoprecipitated with the 145-2C11 (2C11) or SP34 mAb and were analyzed by two-dimensional nonreducing (NR)/reducing (R) SDS/12.5% PAGE. The location of CD3-ε^h (ε^h) and CD3-ε^m (ε^m) is indicated. The molecular mass markers in kDa are indicated on the right.

genic mouse that expresses the CD3-ε^h gene (8). In these mice, the majority of thymocytes expressed both CD3-ε^m and CD3-ε^h chains on their cell surface as determined by two-color immunofluorescence analysis (Fig. 5*A*). Thymocytes were isolated from wild-type and transgenic mouse thymus and surface radiolabeled with ^{125}I. Lysates were immunoprecipitated with the 145-2C11 and SP34 mAbs, and the isolated protein was analyzed by two-dimensional nonreducing/reducing SDS/PAGE (Fig. 5*B*). Both CD3-ε^h and CD3-ε^m subunits were coisolated with either the CD3-ε^m-specific mAb (145-2C11) or the CD3-ε^h-specific mAb (SP34) from thymocytes derived from the transgenic animal (Fig. 5*B Right*) but not the wild-type animal (Fig. 5*B Left*). Similar findings were observed with lymphocytes obtained from transgenic and wild-type peripheral lymph nodes (data not shown). These studies showed that a chimeric receptor containing both CD3-ε^h and CD3-ε^m subunits was present on a significant fraction of cells isolated from transgenic mice.

CD3-ε Subunit Is Able to Form Disulfide-Linked Dimers in Murine and Human T Cells. Taken together, the preceding studies indicated that, in transfected T-cell lines and transgenic mice, TCR–CD3 complexes contained both the CD3-ε^m and CD3-ε^h subunits. To investigate whether the two CD3-ε subunits present in the TCR–CD3 complex interacted with one another, cell surface radioiodinated human and murine T cells were analyzed for the presence of disulfide-linked CD3-ε homodimers. Analysis of several murine T-cell lines indicated that, in addition to a probable CD3-γε disulfide-linked heterodimer (arrows in Fig. 6 *Left*), a protein migrated at the position of a disulfide-linked CD3-ε^m homodimer (ε-ε in Fig. 6 *Left*). Furthermore, as would be expected of the CD3-ε protein, this band represented a nonglycosylated

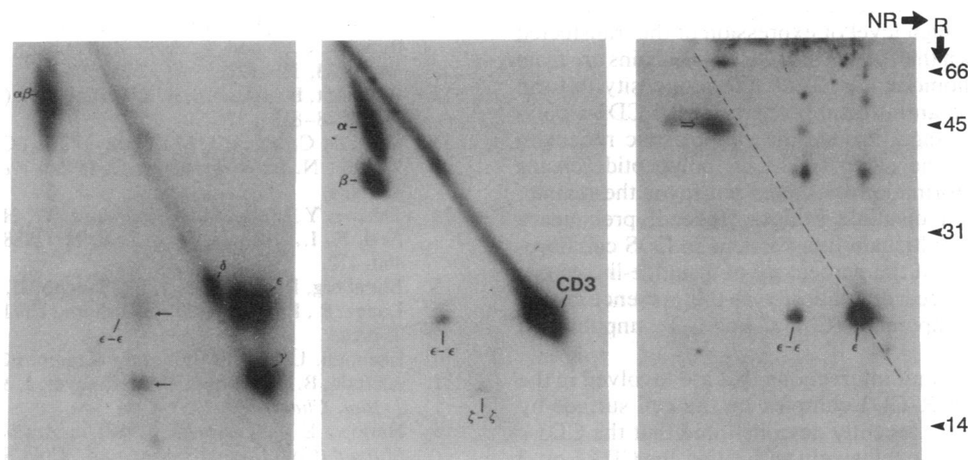

FIG. 6. Identification of CD3-ε disulfide-linked homodimers in human and murine T cells. (*Left* and *Center*) A lysate of ^{125}I-radiolabeled material from the murine T-cell line SL12.4αβ (*Left*) and the human T-cell line HPB-ALL (*Center*) was prepared as outlined, immunoprecipitated with either the 145-2C11 mAb (*Left*) or the Leu-4 mAb (*Center*) and analyzed by two-dimensional nonreducing (NR)/reducing (R) SDS/12.5% PAGE. The location of the components of the TCR–CD3 complex is indicated. ε-ε indicates the location of a CD3-ε homodimer. The arrows indicate the location of a probable CD3-γε heterodimer. (*Right*) Leu-4 immunoprecipitates of the HPB-ALL T-cell line, separated by two-dimensional nonreducing (NR)/reducing (R) SDS/PAGE, were analyzed by Western blotting with the SP34 mAb as described. The location of a CD3-ε monomer (ε) and disulfide-linked homodimer (ε-ε) is indicated. The broken line indicates the location of the diagonal. The double arrow indicates the location of the immunoglobulin heavy chain used in the initial immunoprecipitation.

protein as determined by its resistance to digestion with N-glycanase (data not shown). Similarly, a band that had the correct mobility for a CD3-ε^h homodimer was detected on the surface of human T-cell lines (ε-ε in Fig. 6 *Center*).

To ascertain that the indicated protein band in the human T-cell line represented disulfide-linked CD3-ε homodimers, a Western blotting experiment was performed. TCR–CD3 complexes were isolated from the human leukemic T-cell line HPB-ALL with the Leu-4 mAb, and the protein components were separated by two-dimensional nonreducing/reducing SDS/PAGE. Upon electrophoretic transfer to nitrocellulose filters, the CD3 proteins were detected with a second mAb specific for CD3-ε^h, SP34, combined with ^{125}I-labeled protein A. The previously identified spot was, indeed, a disulfide-linked CD3-ε homodimer (ε-ε in Fig. 6 *Right*). However, densitometric analysis showed that 75% of the CD3-ε protein migrated as a nondisulfide-linked form (ε in Fig. 6 *Right*). We have also detected CD3-ε disulfide-linked homodimers in immunoprecipitates of radioiodinated material from human T cells, using TCR-specific antibodies (data not shown). In conclusion, CD3-ε disulfide-linked homodimers can be observed on the cell surface of human and murine T cells in association with the TCR. Therefore, the CD3-ε polypeptides have a propensity to form homodimeric structures.

DISCUSSION

The results presented in this paper indicated that the TCR–CD3 complex contained two CD3-ε subunits. This conclusion was supported by the use of gene transfer of the CD3-ε^h cDNA and genomic DNA into murine T cells to produce either CD3-ε^h-expressing murine T–T hybridomas or CD3-ε^h-expressing thymocytes in transgenic mice. Analysis of these chimeric T cells using species-specific anti-CD3-ε mAbs demonstrated that the CD3-ε^h protein was expressed in the same TCR–CD3 complex as the CD3-ε^m protein. Immunodepletion experiments indicated that most of the CD3-ε^h protein in all of the transfectant cells was present in a TCR–CD3 complex that also contained the CD3-ε^m polypeptide chain.

The observation that a small fraction of the CD3-ε^h and CD3-ε^m proteins were disulfide-linked on the cell surface further indicated that ε–ε homodimers existed within the TCR–CD3 complex. Although not formally proven, it seems likely that CD3-ε chains that are not disulfide-linked are present as non-disulfide-linked homodimers. Preliminary results with the COS cell fibroblast transfection system, which can achieve a very high level of expression of the transfected protein, have shown that >90% of the CD3-ε^h chains are from disulfide-bridged homodimers. Thus, the propensity to form dimers seems to be an intrinsic property of the CD3-ε polypeptide chains. Passage through the endoplasmic reticulum and assembly with the other TCR–CD3 polypeptide chains may induce a conformation that does not favor the maintenance of interchain disulfide bridges. Indeed, preliminary observations with multichain transfections in COS cell fibroblasts have shown that the percentage of disulfide-linked ε–ε homodimers is reduced dramatically in the presence of the other TCR–CD3 components (C. Hall and C.T., unpublished data).

A study of the subunit interactions that are involved in the formation of the TCR–CD3 complex on the cell surface by Koning *et al.* (19) has recently demonstrated that the CD3-ε subunit may associate exclusively with either the CD3-γ or -δ chains. We have additional evidence to suggest that these $\gamma\varepsilon$ and $\delta\varepsilon$ subcomplexes may, in fact, exist on the cell surface as a part of two distinct TCR–CD3 complexes. One contains only the CD3-γ subunit and the other contains only the CD3-δ subunit (B. A. Alarcon and C.T., unpublished data). These complexes have been defined with antibodies that recognize either the CD3-γ or -δ proteins and consist of either $\alpha\beta\gamma\varepsilon\zeta_2$ (γ-type) or $\alpha\beta\delta\varepsilon\zeta_2$ (δ-type). In view of these findings and our results, which suggest that the TCR–CD3 complex contains two CD3-ε proteins in close proximity to one another, we propose that the TCR–CD3 proteins may form complexes that have a stoichiometry of either $\alpha\beta\gamma\varepsilon\varepsilon\gamma\zeta_2$ or $\alpha\beta\delta\varepsilon\varepsilon\delta\zeta_2$. In addition, since specific interactions between CD3-ε and the TCR-β chain have been described (20), it is tempting to further speculate on the possible existence of either a $\zeta_2\alpha\beta\gamma\varepsilon\varepsilon\gamma\alpha\beta\zeta_2$- or $\zeta_2\alpha\beta\delta\varepsilon\varepsilon\delta\alpha\beta\zeta_2$-containing complex. This latter model predicts, therefore, that the TCR, like immunoglobulins, may be bivalent, with the CD3-ε protein providing a bridge between the two binding sites. Finally, the interaction of CD3-ζ and -η with these various forms of the receptor would create additional subcomplexes that may play distinct roles in regulating T-cell function (21).

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1. Marrack, P. & Kappler, J. (1987) *Science* **238**, 1073–1078.
2. Raulet, R. H. (1989) *Annu. Rev. Immunol.* **7**, 175–207.
3. Clevers, H., Alarcon, B., Wileman, T. & Terhorst, C. (1988) *Annu. Rev. Immunol.* **6**, 629–662.
4. Baniyash, M., Garcias-Morales, P., Bonafacino, J. S., Samelson, L. E. & Klausner, R. D. (1988) *J. Biol. Chem.* **263**, 9874–9879.
5. Sleckman, B. P., Peterson, A., Jones, W. K., Foran, J. A., Greenstein, J. L., Seed, B. & Burakoff, S. J. (1987) *Nature (London)* **328**, 351–353.
6. Marrack, P., Endres, R., Shimonkevitz, R., Zlotnick, L., Dialynas, D., Fitch, F. & Kappler, J. (1983) *J. Exp. Med.* **158**, 1077–1091.
7. Ley, S. C., Tan, K. N., Kubo, R., Sy, M. S. & Terhorst, C. (1989) *Eur. J. Immunol.* **19**, 2309–2317.
8. Clevers, H. C., Lonberg, N., Dunlap, S., Lacy, E. & Terhorst, C. (1989) *EMBO J.* **8**, 2527–2535.
9. Pessano, S., Oettgen, H., Blan, A. K. & Terhorst, C. (1985) *EMBO J.* **4**, 337–344.
10. Leo, O., Foo, M., Sachs, D. H., Samelson, L. E. & Bluestone, J. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1374–1378.
11. Ledbetter, J. A., Evans, R. L., Lipinski, M., Cunningham-Rundles, C., Good, R. A. & Herzenberg, L. A. (1981) *J. Exp. Med.* **153**, 310–323.
12. Berkhout, B., Alarcon, B. & Terhorst, C. (1988) *J. Biol. Chem.* **263**, 8528–8536.
13. Gold, D. C., Puck, J. M., Pettey, C. L., Cho, M., Coligan, J., Woody, N. N. & Terhorst, C. (1986) *Nature (London)* **321**, 431–434.
14. Takebe, Y., Serk, M., Fujisawa, J. Y., Hoy, I., Yokota, K., Arai, K. I., Yoshida, M. & Arai, N. (1988) *Mol. Cell. Biol.* **8**, 460–472.
15. Blumberg, R. S., Alarcon, B., Sancho, J., McDermott, F. V., Lopez, P., Breitmeyer, J. & Terhorst, C. (1990) *J. Biol. Chem.*, in press.
16. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
17. Alarcon, B., Berkhout, B., Breitmeyer, J. & Terhorst, C. (1988) *J. Biol. Chem.* **283**, 2953–2961.
18. Harlow, E. & Lane, D. (1988) in *Antibodies-A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 483–503.
19. Koning, F., Maloy, W. L. & Coligan, J. E. (1990) *Eur. J. Immunol.* **20**, 299–305.
20. Wileman, T., Carson, G. R., Concino, M., Ahmed, A. & Terhorst, C. (1990) *J. Cell Biol.* **110**, 973–986.
21. Mercep, M., Bonifacino, J. S., Garcia-Morales, P., Samelson, L. E., Klausner, R. D. & Ashwell, J. D. (1988) *Science* **242**, 571–574.