Immunity, Vol. 2, 639–644, June, 1995, Copyright © 1995 by Cell Press

Rapid Turnover of the CD3ζ Chain Independent of the TCR-CD3 Complex in Normal T Cells

Satoru Ono, Hiroshi Ohno, and Takashi Saito
Division of Molecular Genetics
Center for Biomedical Science
Chiba University School of Medicine
1-8-1, Inohana, Chuo-ku
Chiba 260
Japan

Summary

The function of CD3ζ in the assembly and transport of the T cell receptor (TCR)-CD3 complex was analyzed in normal T cells. The ζ chain, but not other chains in the surface TCR complex, rapidly exchanged with newly synthesized ζ. Because ζ was expressed independently from the complex, the TCR complex may be transported to the surface along the ζ turnover pathway by association with ζ. These data suggest the dynamic nature of ζ metabolism and provide the evidence that a single component in a multisubunit receptor exhibits independent metabolism from the rest of the complex.

Introduction

The T cell antigen receptor (TCR) is a multisubunit complex composed of at least six different chains; α, β, γ, δ, ε, and ζ (Oettgen et al., 1986; Clevers et al., 1988; Ashwell and Klausner, 1990; Klausner et al., 1990). These components of the TCR–CD3 complex are divided into three groups of dimers; a clonotypic αβ heterodimer, invariant CD3 γε and δε, and a dimer of CD3ζ family molecules that consists of ζ homodimers, ζ plus the η chain or the γ chain of Fc receptor (FcRγ), or FcRγ dimers (Samelson et al., 1985; Baniyash et al., 1988; Orloff et al., 1990; Bauer et al., 1991; Koyasu et al., 1992; Ohno et al., 1994). The ζ chain has two major functions in T cells: transduction of signals after the TCR engages its ligand, which is mediated through the activation motifs in the cytoplasmic region (Malissen and Schmitt-Verhulst, 1993; Aoe et al., 1994; Weiss and Littman, 1994), and regulation of the assembly and intracellular transport of the TCR–CD3 complex (Klausner et al., 1990).

The TCR–CD3 complex of T cell hybridomas is an established model system of assembly and transport of a multisubunit surface receptor complex (Klausner et al., 1990). In this system, all components except for ζ are synthesized in excess and are susceptible to degradation in the endoplasmic reticulum (ER) unless assembled with other chains (Minami et al., 1987a; Chen et al., 1988; Lippincott-Schwartz et al., 1988; Bonifacino et al., 1989; Wileman et al., 1990). Even if individual chains are protected from ER degradation by association with other components, incomplete TCR complexes (αβγδε) that lack ζ are targeted to the lysosome for degradation, and only fully assembled complexes (αβγδεζ) can be transported to the cell surface (Minami et al., 1987a; Sussman et al., 1988; Bonifacino et al., 1989). It has been concluded that only ζ is limiting, and all the ζ synthesized is efficiently assembled with the partially assembled TCR complex to form the complete complex; therefore, ζ determines the rate and fate of the assembly and transport (Minami et al., 1987a; Sussman et al., 1988; Bonifacino et al., 1989; Klausner et al., 1990). These conclusions, however, have been derived exclusively from analyses of T cell hybridomas or tumors. During analysis of the function of the ζ chain in normal T cells, we found that the function of ζ in regulation of the assembly and transport of the TCR–CD3 complex was different in normal T cells from that observed in T cell hybridomas.

Results

Specific Disappearance of CD3ζ in the Surface TCR Complex

To analyze the fate of the TCR–CD3 complex expressed on the cell surface in normal T cells, splenic T cells isolated through a nylon column were surface biotinylated, cultured for various time periods, and lysed. The lysates were immunoprecipitated with monoclonal antibodies (MAbs) to TCRβ, CD3ε, and CD3ζ. Although the amount of TCRαβ and CD3γδε was not affected by incubation, the ζ chain specifically disappeared after 2 hr of incubation. The disappearance of biotinylated ζ was observed by precipitation with all three MAbs (Figure 1). The possibility that the loss of ζ was due to the biotinylation was excluded by the observation that specific loss of ζ was also observed when the ζ chain was surface iodinated rather than biotinylated (Figure 2). We then examined 2B4, a T cell hybridoma (Minami et al., 1987a; Sussman et al., 1988; Bonifacino et al., 1989; Klausner et al., 1990), for comparison, because 2B4 has been analyzed extensively for the assembly and transport of the TCR complex. Whereas splenic T cells lost ζ within 4 hr, the amount of surface ζ in 2B4 did not change; thus, the turnover of surface ζ on normal T cells was different from that on 2B4 (Figure 3). Two-dimensional gel analysis also confirmed that the 16 kDa molecule that disappeared was CD3ζ. We noticed that the MAb to ζ precipitated the TCR–CD3 complex in spite of the disappearance of the biotinylated ζ (see Figure 1), suggesting that although biotinylated ζ disappeared, ζ existed within the surface TCR complex and that the biotinylated ζ was replaced by nonlabeled ζ.

Rapid Turnover of the CD3ζ Chain within the TCR Complex on the Cell Surface

To test this hypothesis, we did three experiments on splenic T cells: surface staining of the TCR complex, biotinylation of the complex after incubation, and Western blotting for total ζ. Surface staining demonstrated that the amount of TCR–CD3 complex remained constant during incubation (Figure 4A). When cells were biotinylated and incubated, ζ disappeared (Figure 4B). However, a normal

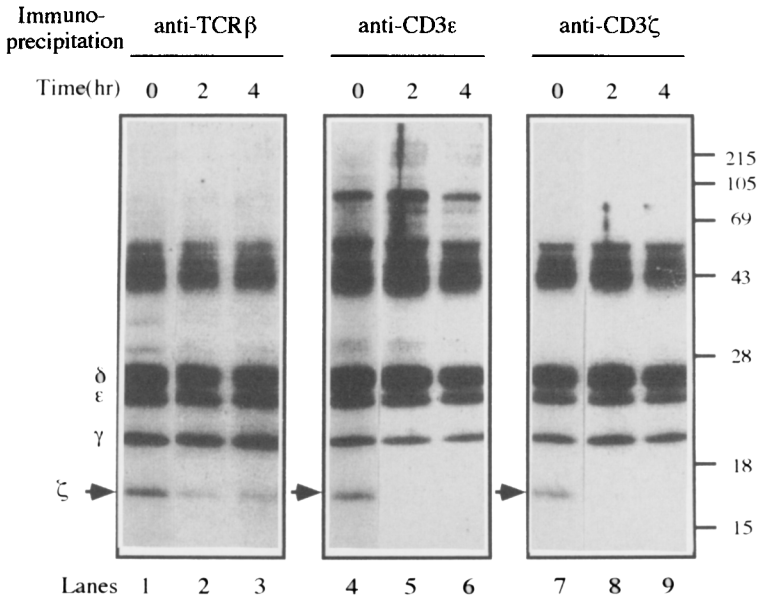


Figure 1. Selective Disappearance of the Biotinylated ζ Chain During Incubation

Splenic T cells were surface biotinylated and incubated for 2 hr (lanes 2, 5, 8), 4 hr (lanes 3, 6, 9), or unincubated (lanes 1, 4, 7). The lysates were immunoprecipitated with MAbs to TCRβ (H57–597) (lanes 1–3), CD3ε (145–2C11) (lanes 4–6), or ζ (H146–968) (lanes 7–9). The bands corresponding to CD3γ, δ, ε, and ζ are indicated.

amount of surface ζ was observed by rebiotinylation after the incubation. Rebiotinylated ζ disappeared again after further incubation. Immunoblotting showed that although biotinylated ζ disappeared, the total amount of ζ remained constant after incubation (Figure 4C). These results indicate that the surface ζ disappeared spontaneously by incubation at 37°C and that only ζ, but not other chains, was rapidly replaced by new ζ within the TCR complex, whereas amounts of TCRαβ–CD3γδε remained constant on the cell surface.

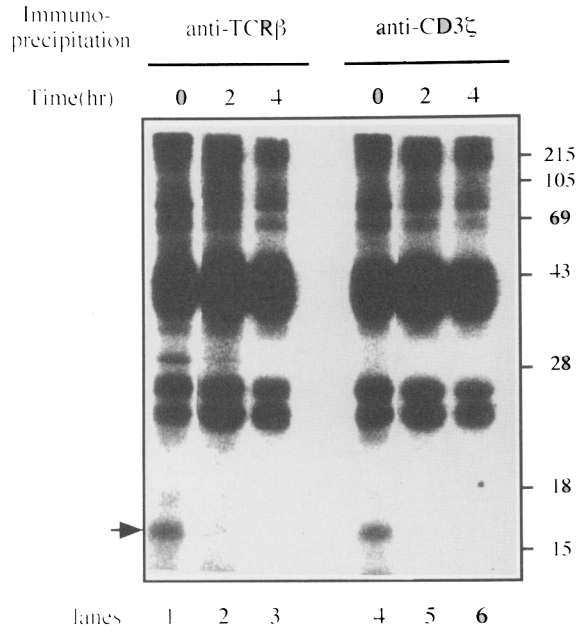


Figure 2. Selective Disappearance of the Surface-Iodinated ζ Chain by Incubation

Splenic T cells were surface iodinated and incubated for 2 and 4 hr. The lysates were precipitated with anti-CD3ζ (H146–968) or anti-TCRβ MAb (H57–597). CD3γ was not observed because it was hardly iodinated.

Internalized CD3ζ Is Targeting for Degradation

Because we did not detect ζ in the culture supernatant after incubation, the ζ replaced by new ζ may have been degraded after internalization. Therefore, we tested several reagents and conditions to inhibit the degradation (Figure 5). Treatments with ammonium chloride, known to block lysosomal degradation (Poole and Ohkuma, 1981), inhibited the disappearance of ζ. Similar results were obtained by treatment with chloroquine (data not shown). The disappearance was completely blocked when cells were incubated at 0°C instead of 37°C. Collectively, the surface labeled ζ was spontaneously internalized and most of the internalized ζ is probably degraded in the lysosomal compartment.

Rapid Turnover of CD3ζ Takes Place in the Absence of the Surface TCR–CD3 Complex

Since ζ exhibits rapid and independent turnover from the TCR–CD3 complex, it is postulated that ζ might be expressed and turned over even in the absence of the surface TCR complex. To this end, we analyzed a surface TCR–CD3-negative mutant of the Jurkat line, J.RT-T3.1 that is deficient in the TCRα chain (Weiss and Stobo, 1984; Gunter et al., 1987). This is based on our findings that human peripheral T cells exhibit ζ turnover similarly to mouse splenic T cells (data not shown) and that only Jurkat cell line exhibits ζ turnover as normal T cells (Figure 6), despite of the fact that murine T cell hybridomas are all resistant for this phenomenon. The absence of the TCR–CD3 complex on the cell surface of J.RT-T3.1 was confirmed by FACS analysis (data not shown) (Saito et al., 1987). As expected, ζ, but not any other TCR–CD3 chains, was detected on the cell surface of the mutant cells (Figure 6). This ζ was not associated with any other biotinylated

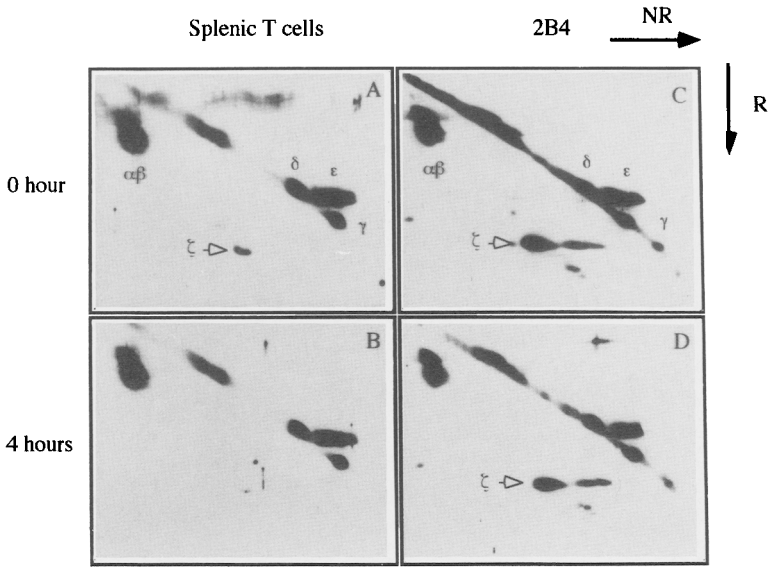
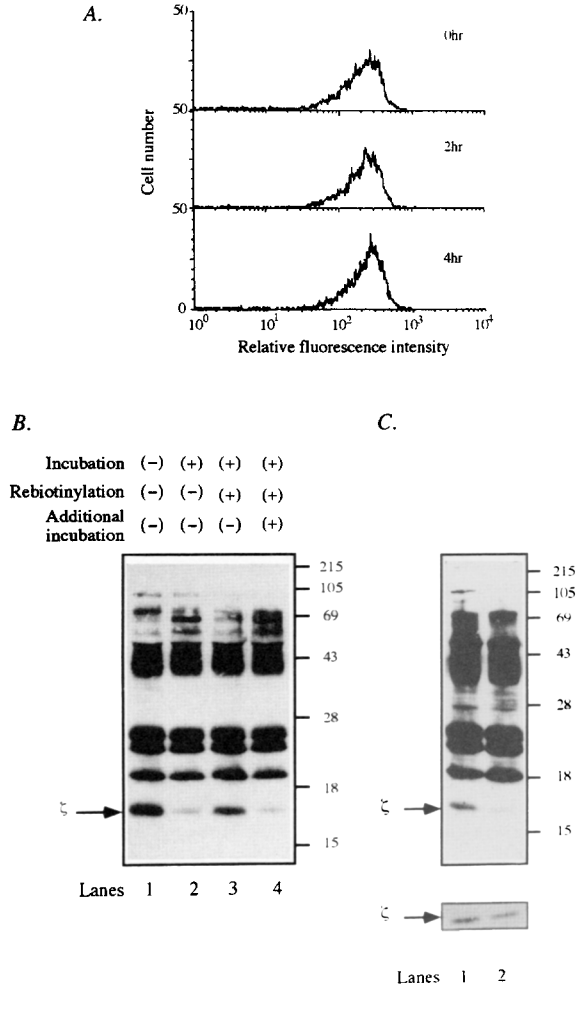


Figure 3. Specific Disappearance of ζ Was Observed in Normal T Cells but Not in 2B4 Cells

Splenic T cells (A and B) and 2B4 (C and D) were surface biotinylated, and lysed after 4 hr incubation (B and D) or without incubation (A and C). The lysates were immunoprecipitated with MAb to ζ (H146–968). The spots corresponding to CD3γ, δ, ε, and ζ are indicated.



molecules. The ζ on the cell surface of these mutant cells disappeared upon incubation, similar to the observation in the wild-type Jurkat and normal T cells (Figure 6). These results indicate that the turnover of the surface ζ chain takes place independently of the surface TCR complex.

Discussion

Our findings provide new insights regarding the assembly and transport of the TCR–CD3 complex. In this system, a single component of a multichain receptor was found to have a different metabolism, independent of the rest of the receptor complex. This observation could be extended to other multichain receptor systems. Our analysis on normal T cells results in a revision of the conclusions from studies on T cell hybridomas (Minami et al., 1987a; Sussman et al., 1988; Bonifacino et al., 1989; Klausner et al.,

Figure 4. Rapid Turnover of the ζ Chain within the TCR Complex on the Cell Surface

(A) Flow cytometric analysis of the cell surface TCR–CD3 complex during incubation at 37°C. Biotinylated splenic T cells were stained with MAb to CD3ε after culture for 2 hr, 4 hr, or without culture.

(B) Surface ζ was replaced by newly transported ζ. Splenic T cells were biotinylated and lysed after 4 hr incubation (lane 2) or without incubation (lane 1). The biotinylated T cells were rebiotinylated after 4 hr incubation and lysed immediately (lane 3) or after an additional 4 hr incubation (lane 4). The lysates were immunoprecipitated with MAb to ζ (H146–968).

(C) Total amount of ζ was constant during incubation. Surface-biotinylated splenic T cells were lysed immediately (lane 1) or after 4 hr culture (lane 2). The lysates were immunoprecipitated with anti-CD3ζ MAb (H146–968). (Top) the biotinylated proteins were detected. (Bottom) the same immunoprecipitated samples were blotted with MAb to ζ (H146–968).

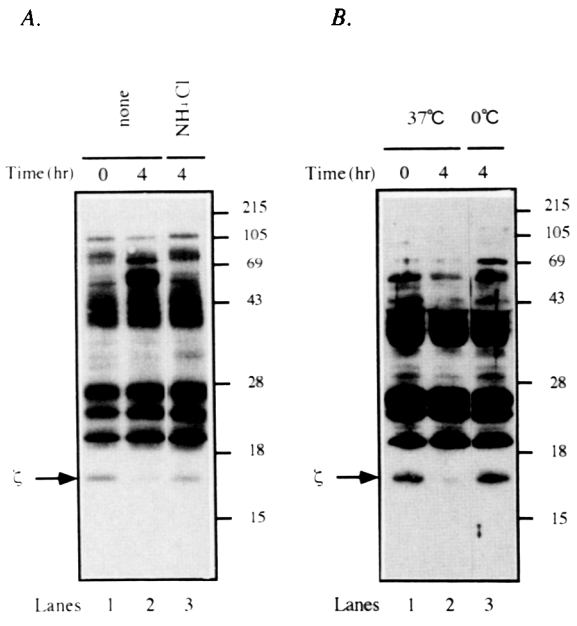


Figure 5. Preventing the Degradation of the Internalized ζ by Various Reagents and Conditions

(A) The effect of ammonium chloride. Splenic T cells were first cultured for 30 min in the presence of 50 mM ammonium chloride (lane 3).

(B) The effect of incubation temperature at 37°C (lane 2) or 0°C (lane 3). All lysates were immunoprecipitated with anti-ζ MAb (H146–968).

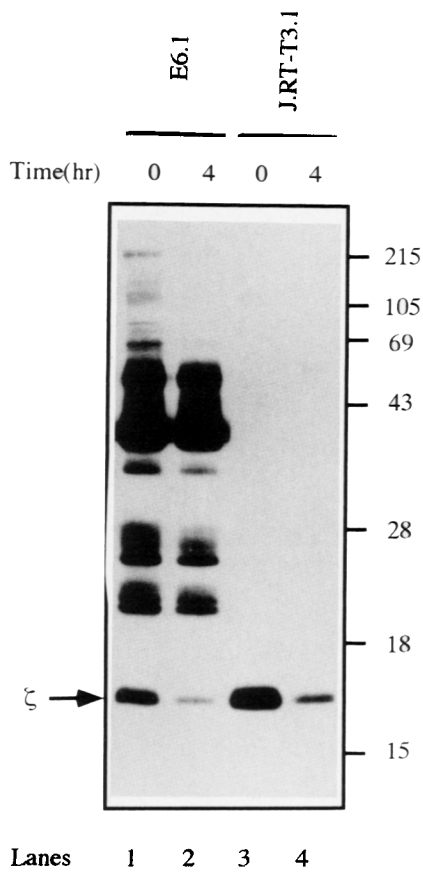


Figure 6. Surface Expression of ζ on a Surface TCR-Negative Mutant T Cell Line

Cells (1 × 10^7) of the wild-type Jurkat line, E6.1 (lanes 1, 2) or a TCRα-deficient Jurkat mutant line, J.RT-T3.1 (lanes 3, 4) were surface biotinylated, lysed immediately (lanes 1, 3) or after 4 hr incubation (lanes 2, 4), immunoprecipitated with MAb to ζ (H146–968).

1990) that only the CD3ζ chain is minimally produced and determines the assembly and the surface expression of the TCR complex, and also that only the completely assembled complex can be transported to the surface. First, ζ on the cell surface is spontaneously replaced by newly transported ζ and internalized without altering the expression of other components of the TCR complex, and the internalized ζ is targeted for lysosomal degradation. Second, ζ can be transported to the cell surface independently of the TCR complex, even in the absence of an association with the TCR complex. In addition, there seems to be a larger pool of ζ than previously thought, from which ζ may be transported independently of the TCR complex, because the surface ζ increased by blocking protein synthesis without affecting the amount of other TCR–CD3 chains on the cell surface (S. O. and T. S., unpublished data). Collectively, the ζ chain exhibits turnover independent of other components in the TCR–CD3 complex, despite its being a component of the TCR complex.

Analysis of ζ-deficient mice (Liu et al., 1993; Love et al., 1993; Malissen et al., 1993; Love et al., 1993 Ohno et al., 1993a) and ζ-variant T cell lines (Weissman et al., 1989; Ohno et al., 1993b) showed that ζ is crucial for the surface expression of the TCR complex. Although ζ possesses its own turnover pathway independent of the TCR complex, the TCR complex requires ζ to be transported to the cell surface. Therefore, it seems that TCRαβ–CD3γδε is transported along the ζ turnover pathway by association with ζ in order to be expressed on the cell surface. Once expressed, ζ exists within the TCR complex but is readily replaced by newly transported ζ, supporting the idea that ζ within the complex exhibits association–dissociation dynamics.

These results show that ζ possesses a specialized regulatory function for the transport and assembly of the TCR–CD3 complex, which may resemble the invariant chain (Ii) for assembly and transport of MHC class II molecules (Cresswell, 1994; Germain, 1994). Newly synthesized class II α and β chains are associated with BiP and calnexin and dissociated by forming the αβ–Ii complex, that is transported to endosomes where Ii dissociates from αβ and is replaced by an antigen peptide. Similarly, TCR-associated protein (TRAP) (Alarcon et al., 1988; Bonifacino et al., 1988) and calnexin (Rajagopalan et al., 1994) have been shown to be associated with newly synthesized unassembled TCR and CD3 chains in ER, and to dissociate after the partial TCRαβ–CD3 complex is assembled. The function of ζ observed in the present study is probably involved in the subsequent transport to the cell surface along the ζ metabolic pathway after ζ associates with the partial TCR–CD3 complex and, subsequently, only ζ dissociates from the complex and internalizes for degradation.

Triggering of the TCR–CD3 complex can lead to its modulation from the cell surface (Krangel, 1987; Minami et al., 1987b; Luton et al., 1994). Considering that the spontaneous exchange and internalization of surface ζ is hardly observed in T cell hybridomas and that T cell hybridomas are resistant to TCR modulation by cross-linking with antibodies, the mechanism of such modulation may include the ζ turnover observed in normal T cells. Furthermore, it has recently been shown that the ζ chain disappears in T cells from tumor-bearing mice and cancer patients, and this appears to be greatly attributable for immunosuppression under these conditions (Mizoguchi et al., 1992; Finke et al., 1993; Nakagomi et al., 1993; Gunji et al., 1994; Aoe et al., 1995). The mechanism for the specific disappearance of ζ without altering other components might be related to the observed metabolism of ζ; the inhibition of the independent transport of ζ might cause the loss of ζ. Thus, molecular analysis of the mechanism of the turnover of ζ independent of other components of the TCR complex could also elucidate the mechanism of immunosuppression under disease conditions.

Experimental Procedures

Cell Preparation

Splenocytes from BALB/c mice (Japan SLC, Incorporated, Hamamatsu, Japan) were depleted of erythrocyte by lysis and T cells were enriched through a nylon column. The murine T cell hybridoma 2B4, the human T cell line Jurkat E6.1, and the TCRα-deficient Jurkat mutant line J.RT-T3.1, were maintained in RPMI 1640 (GIBCO, Grand Island, New York) supplemented with 5% heat-inactivated fetal bovine serum, 10 mM HEPES (pH 7.6), and 100 μg/ml kanamycin.

Cell Surface Biotinylation, Immunoprecipitation, and SDS-PAGE Analysis

Cells were surface biotinylated as previously described (Ohno et al., 1994). In brief, cells were washed three times with phosphate-buffered saline, and were suspended in labeling buffer containing 10 mM HEPES (pH 8.0), 150 mM NaCl at a concentration of 1×10^7 cells/ml. Biotin-XX (10 mg/ml), succinimidyl ester (Molecular Probes, Incorporated, Eugene, Oregon), dissolved in DMSO, were added to the cell suspension at final concentration of 100 μg/ml, then incubated for 1 hr on ice with occasional shaking. Biotinylated cells were washed twice with RPMI 1640, incubated at 37°C for 2 or 4 hr or without incubation, in RPMI 1640 supplemented 10% heat-inactivated fetal calf serum, 10 mM HEPES (pH 7.6), 2 mM glutamine, 50 μM 2-ME, and 100 μg/ml kanamycin, and then washed twice with RPMI 1640. Cells were lysed with a lysis buffer containing 1% digitonin, 50 mM Tris–HCl (pH 7.6), 150 mM NaCl, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM PMSF, 10 mM iodoacetamide, at a concentration of 1×10^7 cells/ml. Immunoprecipitation was performed with anti-CD3ζ MAb (H146–968), anti-CD3ε MAb (145–2C11), or anti-TCRβ MAb (H57–597). Immunoprecipitates were seperated either on single-dimensional SDS–14% polyacrylamide gel electrophoresis (PAGE) under reducing condition, or on two-dimentional nonreducing (12%) and reducing (14%) SDS–PAGE and transferred onto a PVDF membrane (Immobilon-P; Millipore Corporation, Bedford, Massachusetts). The biotinylated proteins were detected using streptavidin–peroxidase (VECTASTAIN Elite ABC kit; Vector Laboratories Incorporated, Burlingame, California), an enhanced chemiluminescence system (Amersham International, Buckinghamshire, England), and autoradiography.

Radioiodination

Splenic T cells (4×10^7) were surface iodinated by lactoperoxidase-catalyzed method with lactoperoxidase (Sigma Chemical Company, St. Louis, Missouri), 0.05% H_2O_2, and 37 MBq of $Na^{125}I$ (Amersham). The cell lysates were prepared, immunoprecipitated, and analyzed on SDS–PAGE as described above. Radioiodinated proteins in the dried gel were detected by Image analyzer (BAS2000; Fuji).

Flow Cytometry

Splenic T cells (1×10^6) were stained with fluorescein isothiocyanate–labeled anti-CD3ε (145-2C11) MAb (PharMingen, San Diego, California). Cells (1×10^4) were analyzed by FACScan flow cytometer (Beckton Dickinson and Company, Mountain View, California). Dead cells were excluded by staining with propidium iodide. Cells in the lymphocyte gate defined by light scatter were collected.

Western Blotting

After proteins were transferred from SDS–PAGE gels to PVDF membranes, the membranes were blocked in 10% skim milk–containing buffer (Block Ace; Yukijirushi, Sapporo, Japan), incubated with 10 μg/ml of anti-CD3ζ MAb (H146-968) in TBS-T (0.2% Tween 20, 20mM Tris–HCl [pH 7.6], 140 mM NaCl). Proteins were visualized by incubation with peroxidase-conjugated rabbit anti-mouse immunoglobulin antibodies (Amersham) and an enhanced chemiluminescence system (Nakano et al., 1994).

Acknowledgments

We thank Dr. R. Kubo for anti-CD3ζ MAb; Drs. T. Aoe, H. Arase and R. N. Germain for helpful discussion; Ms. C. Ogata for technical assistance; and Ms. H. Yamaguchi for preparation of the manuscript. Supported by grants from the Ministry for Education, Science and Culture, and from the Agency for Science and Technology, Japan.

Received January 17, 1995; revised March 28, 1995.

References

Alarcon, B., Berkhout, B., Breitmeyer, J., and Terhorst, C. (1988). Assembly of the human T cell receptor–CD3 complex takes place in the endoplasmic reticulum and involves intermediary complexes between the CD3-γ·δ·ε core and single T cell receptor α or β chains. J. Biol. Chem. *263*, 2953–2961.

Aoe, T., Goto, S., Ohno, H., and Saito, T. (1994). Different cytoplasmic structure of the CD3ζ family dimer modulates the activation signal and function of T cells. Int. Immunol. *6*, 1671–1679.

Aoe, T., Okamoto, Y., and Saito, T. (1995). Activated macrophages induce structural abnormalities of the T cell receptor–CD3 complex. J. Exp. Med. *181*, 1881–1886.

Ashwell, J. D., and Klausner, R. D. (1990). Genetic and mutational analysis of the T-cell antigen receptor. Annu. Rev. Immunol. *8*, 139–167.

Baniyash, M., Garcia-Morales, P., Bonifacino, J. S., Samelson, L. E., and Klausner, R. D. (1988). Disulfide linkage of the ζ and η chains of the T cell receptor. J. Biol. Chem. *263*, 9874–9878.

Bauer, A., McConkey, D. J., Howard, F. D., Clayton, L. K., Novick, D., Koyasu, S., and Reinherlz, E. L. (1991). Differential signal transduction via T-cell receptor $CD3\zeta_2$, $CD3\zeta$-η, and $CD3\eta_2$ isoforms. Proc. Natl. Acad. Sci. USA *88*, 3842–3846.

Bonifacino, J. S., Lippincott-Schwartz, J., Chen, C., Antusch, D., Samelson, L. E., and Klausner, R. D. (1988). Association and dissociation of the murine T cell receptor associated protein (TRAP). J. Biol. Chem. *263*, 8965–8971.

Bonifacino, J. S., Suzuki, C. K., Lippincott-Schwartz, J., Weissman, A. M., and Klausner, R. D. (1989). Pre-Golgi degradation of newly synthesized T-cell antigen receptor chains: intrinsic sensitivity and the role of subunit assembly. J. Cell Biol. *109*, 73–83.

Chen, C., Bonifacino, J. S., Yuan, L. C., and Klausner, R. D. (1988). Selective degradation of T cell antigen receptor chains retained in a pre-Golgi compartment. J. Cell Biol. *107*, 2149–2161.

Clevers, H., Alarcon, B., Wileman, T., and Terhorst, C. (1988). The T cell receptor/CD3 complex: a dynamic protein ensemble. Annu. Rev. Immunol. *6*, 629–662.

Cresswell, P. (1994). Assembly, transport, and function of MHC class II molecules. Annu. Rev. Immunol. *12*, 259–293.

Finke, J. H., Zea, A. H., Stanley, J., Longo, D. L., Mizoguchi, H., Tubbs, R. R., Wiltrout, R. H., O'Shea, J. J., Kudoh, S., Klein, E., Bukowski, R. M., and Ochoa, A. C. (1993). Loss of T-cell receptor ζ chain and $p56^{lck}$ in T-cells infiltrating human renal cell carcinoma. Cancer Res. *53*, 5613–5616.

Germain, R. N. (1994). MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell *76*, 287–299.

Gunji, Y., Hori, S., Aoe, T., Asano, T., Ochiai, T., Isono, K., and Saito, T. (1994). High frequency of cancer patients bearing abnormal assembly of the T cell receptor–CD3 complex in peripheral blood T lymphocytes. Jpn. J. Cancer Res. *85*, 1189–1192.

Gunter, K. C., Germain, R. N., Kroczek, R. A., Saito, T., Yokoyama, W. M., Chan, C., Weiss, A., and Shevach, E. M. (1987). Thy-1–mediated T-cell activation requires co-expression of CD3/Ti complex. Nature *326*, 505–507.

Klausner, R. D., Lippincott-Schwartz, J., and Bonifacino, J. S. (1990). The T cell antigen receptor: insights into organelle biology. Annu. Rev. Cell Biol. *6*, 403–431.

Koyasu, S., D'Adamio, L., Arulanandam, A. R. N., Abraham, S., Clayton, L. K., and Reinherz, E. L. (1992). T cell receptor complexes containing FcεRIγ homodimers in lieu of CD3ζ and CD3η components: a novel isoform expressed on large granular lymphocytes. J. Exp. Med. *175*, 203–209.

Krangel, M. S. (1987). Endocytosis and recycling of the T3–T cell receptor complex. J. Exp. Med. *165*, 1141–1159.

Liu, C. P., Ueda, R., She, J., Sancho, J., Wang, B., Weddell, G., Loring, J., Kurahara, C., Dudley, E. C., Hayday, A., Terhorst, C., and Huang, M. (1993). Abnormal T cell development in CD3-$\zeta^{-/-}$ mutant mice and identification of a novel T cell population in the intestine. EMBO J. *12*, 4863–4875.

Love, P. E., Shores, E. W., Johnson, M. D., Tremblay, M. L., Lee, E. J., Grinberg, A., Huang, S. P., Singer, A., and Westphal, H. (1993). T cell development in mice that lack the ζ chain of the T cell antigen receptor complex. Science *261*, 918–921.

Luton, F., Buferne, M., Davoust, J., Schmitt-Verhulst, A.-M., and Boyer, C. (1994). Evidence for protein tyrosine kinase involvement in ligand-induced TCR/CD3 internalization and surface redistribution. J. Immunol. *153*, 63–72.

Lippincott-Schwartz, J., Bonifacino, J. S., Yuan, L. C., and Klausner, R. D. (1988). Degradation from the endoplasmic reticulum: disposing of newly synthesized proteins. Cell *54*, 209–220.

Malissen, B., and Schmitt-Verhulst, A.-M. (1993). Transmembrane signaling through the T-cell-receptor–CD3 complex. Curr. Opin. Immunol. *5*, 324–333.

Malissen, M., Gillet, A., Rocha, B., Trucy, J., Vivier, E., Boyer, C., Köntgen, F., Brun, N., Mazza, G., Spanopoulou, E., Guy-Grand, D., and Malissen, B. (1993). T cell development in mice lacking the CD3-ζ/η gene. EMBO. J. *12*, 4347–4356.

Minami, Y., Weissman, A. M., Samelson, L. E., and Klausner, R. D. (1987a). Building a multichain receptor: synthesis, degradation, and assembly of T-cell antigen receptor. Proc. Natl. Acad. Sci. USA *84*, 2688–2692.

Minami, Y., Samelson, L. E., and Klausner, R. D. (1987b). Internalization and cycling of the T cell antigen receptor. J. Biol. Chem. *262*, 13342–13347.

Mizoguchi, H., O'Shea, J. J., Longo, D. L., Loeffler, C. M., McVicar, D.W., and Ochoa, A. C. (1992). Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. Science *258*, 1795–1798.

Nakagomi, H., Petersson, M., Magnusson, I., Juhlin, C., Matsuda, M., Mellstedt, H., Taupin, J.-L. Vivier, E., Anderson, P., and Kiessling, R. (1993). Decreased expression of the signal-transducing ζ chains in tumor-infiltrating T-cells and NK cells of patients with colorectal carcinoma. Cancer Res. *53*, 5610–5612.

Nakano, H., Ohno, H., and Saito, T. (1994). Activation of phospholipase C-γ1 through transfected platelet-derived growth factor receptor enhances interleukin 2 production upon antigen stimulation in a T-cell line. Mol. Cell. Biol. *14*, 1213–1219.

Oettgen, H. C., Pettey, C. L., Maloy, W. L., and Terhorst, C. (1986). A T3-like protein complex associated with the antigen receptor on murine T cells. Nature *320*, 272–275.

Ohno, H., Aoe, T., Taki, S., Kitamura, D., Ishuda, Y., Rajewsky, K., and Saito, T. (1993a). Developmental and functional impairment of T cells in mice lacking CD3ζ chains. EMBO J. *12*, 4357–4366.

Ohno, H., Aoe, T., Ra, C., Yamamoto, T., and Saito, T. (1993b). TCR isoform containing the Fc receptor γ chain exhibits structural and functional differences from isoform containing CD3ζ. Int. Immunol. *5*, 1403–1411.

Ohno, H., Ono, S., Hirayama, N., Shimada, S., and Saito, T. (1994). Preferential usage of the Fc receptor γ chain in the T cell antigen receptor complex by γ/δ T cells localized in epithelia. J. Exp. Med. *179*, 365–369.

Orloff, D. G., Ra, C., Frank, S. J., Klausner, R. D., and Kinet, J. P. (1990). Family of disulfide-linked dimers containing the ζ and η chains of the T-cell receptor and the γ chain of Fc receptors. Nature *347*, 189–191.

Poole, B., and Ohkuma, S. (1981). Effect of weak bases on the intralysosomal pit in mouse peritoneal macrophages. J. Cell Biol. *90*, 665–669.

Rajagopalan, S., Yu, Y., and Brenner, M. B. (1994). Retention of unassembled components of integral membrane proteins by calnexin. Science *263*, 387–390.

Saito, T., Weiss, A., Gunter, K. C., Shevach, E. M., and Germain, R. N. (1987). Cell surface T3 expression requires the presence of both α- and β-chains of the T cell receptor. J. Immunol. *139*, 625–628.

Samelson, L. E., Harford, J. B., and Klausner, R. D. (1985). Identification of the components of the murine T cell antigen receptor complex. Cell *43*, 223–231.

Sussman, J. J., Bonifacino, J. S., Lippincott-Schwartz, J., Weissman, A. M., Saito, T., Klausner, R. D., and Ashwell, J. D. (1988). Failure to synthesize the T cell CD3-ζ chain: structure and function of a partial T cell receptor complex. Cell *52*, 85–95.

Weiss, A., and Stobo, J. D. (1984). Requirement for the coexpression of T3 and the T cell antigen receptor on a malignant human T cell line. J. Exp. Med. *160*, 1284–1299.

Weiss, A., and Littman, D. R. (1994). Signal transduction by lymphocyte antigen receptors. Cell *76*, 263–274.

Weissman, A. M., Frank, S. J., Orloff, D. G., Mercep, M., Ashwell, J.D., and Klausner, R. D. (1989). Role of the zeta chain in the expression of the T cell antigen receptor: genetic reconstitution studies. EMBO. J. *8*, 3651–3656.

Wileman, T., Carson, G. R., Concino, M., Ahmed, A., and Terhorst, C. (1990). The γ and ε subunits of the CD3 complex inhibit pre-Golgi degradation of newly synthesized T cell antigen receptors. J. Cell Biol. *110*, 973–986.