Specific Interaction of Topoisomerase IIβ and the CD3ε Chain of the T Cell Receptor Complex*

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T cell antigen receptor (TCR)-CD3 complex is composed of six different subunits: TCRα and TCRβ and CD3γ, CD3δ, CD3ε, and CD3ζ. Antigen recognition signals are transduced from TCR to the cytoplasm through the cytoplasmic domain of the CD3 chains. To understand the downstream signal transduction pathways, we cloned genes encoding proteins capable of binding to CD3ε with a probe of glutathione S-transferase fused to the cytoplasmic region of CD3ε. One of these clones was found to encode topoisomerase IIβ (topoIIβ). The binding region of CD3ε is located within the N-terminal 12 amino acids containing the motif of a basic amino acid cluster. A similar motif was found in the γ chain of Fc receptors (FcRγ) but not in the CD3ε chain, and indeed, FcRγ but not CD3ε bound to topoIIβ. The binding region of topoIIβ was determined to be the C terminus. Since this region appears to be the regulatory region of the enzymatic activity, the binding of CD3ε might affect the function of topoIIβ. Although topoIIβ is localized mainly in the nucleus and CD3ε is a membrane protein, we demonstrated the presence of CD3ε in the nuclear fraction of thymocytes, which increased upon T cell activation. The specific interaction in cells was evidenced by co-immunoprecipitation of topoIIβ and CD3ε from the nuclear fraction of T cells. The possible function of this interaction is discussed.

The T cell antigen receptor (TCR)–CD3 complex is composed of six subunits: clonotypic α and β chains and invariant CD3γ, CD3δ, CD3ε, and CD3ζ chains. TCRα and TCRβ chains recognize antigen in association with major histocompatibility complex molecules, and the CD3 chains are responsible for transducing antigen recognition signals by TCR from the membrane to the cytoplasm. CD3γ, CD3δ, CD3ε, and CD3ζ contain a conserved amino acid sequence motif, ITAM (immunoreceptor tyrosine-based activation motif) in their cytoplasmic domain (1), which is composed of a pair of the YXXLΦ sequence with a spacer of 7–8 amino acids. ITAM is also present in the cytoplasmic tail of Igα and Igγ of the B cell antigen receptor complex and the γ chain of Fc receptors (FcRγ) (2). Since the TCR-CD3 complex does not possess any intrinsic kinase function, tyrosine kinases associated with the complex have been shown to be important for signal transduction. Fyn and Lck associate noncovalently with the TCR-CD3 complex and CD4/CD8, respectively (3, 4). Upon TCR stimulation, these kinases are activated and phosphorylate several cellular substrates (5). Two tyrosine residues within ITAM are also phosphorylated and become the binding site of the SH2 domains of ZAP-70 or Syk kinase (6, 7). In T cells, the recruitment of ZAP-70 to CD3ε or CD3ζ (8, 9) induces the activation of this kinase and subsequently exhibits functions such as the production of lymphokines.

Utilizing chimeric molecules such as CD8ε or Tacε (the extracellular domain of CD8 or the α chain of the IL-2 receptor, fused to the cytoplasmic domain of ε or ε, respectively) (10, 11), it has been demonstrated that each of the cytoplasmic domains of CD3ε chains induces similar activation events to those through the TCR-CD3 complex, including tyrosine phosphorylation, Ca2+ mobilization, and IL-2 production. On the other hand, there is evidence to indicate that the TCR complex is composed of two activation modules, CD3γε and CD3ζ (12, 13). Molecules important for signaling pathways associated with each of the activation modules have to be determined. Signals mediated through the ε chain are required for some activation such as Thy-1 (12, 13) and CD2-mediated stimulation (14). The relationship between signals through these two modules has not yet been clarified. In addition, it has been shown that stimulation through CD3ε of immature thymocytes without undergoing rearrangement of TCR genes induces differentiation of thymocytes, indicating that signals through CD3ε play important roles in T cell development (15, 16). Furthermore, although phosphorylation-dependent signals in T cells have been extensively analyzed, the molecules associated with unphosphorylated forms of CD3ε or CD3ζ other than Fyn have not yet been identified (3, 17).

In order to understand the downstream signaling events through CD3ε, one of the TCR activation modules, we cloned genes encoding CD3ε-binding proteins. One of these clones was found to encode topoisomerase IIβ (topoIIβ) (18). We demonstrated the presence of CD3ε in the nuclear fraction upon T cell activation and showed the evidence of the specific association in vivo between CD3ε and topoIIβ in this fraction of T cells.

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1. The abbreviations used are: TCR, T cell receptor; aa, amino acid; FcRγ, the γ chain of Fc receptors; GST, glutathione S-transferase; Ig, immunoglobulin; IPTG, isopropyl-β-D-thiogalactopyranoside; ITAM, immunoreceptor tyrosine-based activation motif; mAb, monoclonal antibody; NLS, nuclear localization signal; PAGE, polycrylamide gel electrophoresis; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PMSF, phenylmethylsulfonyl fluoride; PVDF, polyvinylidene difluoride; topoI, topoisomerase II; HBB, Hepes balanced buffer; IL, interleukin.
Detailed mapping of the binding regions of both CD3ε and topoIIβ indicates that the association depends on a novel motif in CD3ε and suggests that the binding might modulate the in vivo function of topoIIβ.

**MATERIALS AND METHODS**

**Cell Culture**—The murine T cell hybridoma 2B4 (19) and DO11.10 (18) were grown in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% fetal calf serum, 5 mM glucose, 50 μM 2-mercaptoethanol, and 100 μg/ml kanamycin. Cos7 cells, designated ED1, ED3, E4 and ED5, were prepared by using pGEX-2TK vector. The pGEX-2TK was kindly provided by Dr. R. Kubo (Cytel Inc., La Jolla, CA), respectively. Anti-μ-galactosidase antibody (Life Technologies, Inc.) and peroxidase-conjugated anti-mouse immunoglobulin (Amersham International, Buckinghamshire, United Kingdom), anti-tubulin antibody (Seikagaku Corp., Tokyo, Japan) and a polyclonal goat anti-hamster Ab (GAH) (Organon Teknika-Cappel) were from commercial sources.

**Construction of GST Fusion Proteins—**GST fusion protein constructs were grown in Escherichia coli and used to gluthathione-Sepharose beads. The beads were washed once with a kinase buffer (20 mM Tris (pH 8.0), 1 mM MgCl2, 0.5 mM dithiothreitol, and the pair of primers to prepare the insert by polymerase chain reaction (PCR): pGEX-2TK-ED1 (aa CD3ε 135–169), E5 and E3; pGEX-2TK-ED3 (aa CD3ε 135–169), E5 and ED3; pGEX-2TK-ED4 (aa CD3ε 147–189), E3 and E5; pGEX-2TK-ED5 (aa CD3ε 135–189), E5 and E5; pGEX-2TK-ED1 (aa CD3ε 135–169), E5 and E5; pGEX-2TK-ED3 (aa CD3ε 135–169), E5 and E3; pGEX-2TK-ED4 (aa CD3ε 147–189), E3 and E5; pGEX-2TK-ED5 (aa CD3ε 135–189), E3 and E5; pGEX-2TK-ED1 (aa CD3ε 135–169), E5 and E5; pGEX-2TK-ED3 (aa CD3ε 135–169), E5 and E3; pGEX-2TK-ED4 (aa CD3ε 147–189), E5 and E5; pGEX-2TK-ED5 (aa CD3ε 135–189), E5 and E5; pGEX-2TK-cDNA (HBB) (20 mM Hepes (pH 7.5), 5 mM MgCl2, 1 mM KCl) and renatured as described previously (21). The membrane were blocked in the HBB buffer containing 5% dry milk (Yukijirushi Ltd., Hokkaido, Japan) at 4°C for 1 h and then probed with 1% dry milk solutions were added at approximately 5 × 10^4 cpm/ml to HBB and incubated overnight. The filters were then washed three times with phosphate-buffered saline (PBS) containing 0.2% Triton X-100 at 4°C, dried, and exposed at −80°C. Positive phases were subsequently isolated and the cDNA inserts were sequenced after subcloning into pBluescript.

**DNA Sequencing—**Double-stranded or single-stranded DNA sequencing was performed using Sequenase 2.0 (U. State Biotechnology, Inc.) and BcaBEST® (Takara, Shiga, Japan) according to the manufacturer’s instructions.

**Filter Binding Experiments—**The lysogens from the positive phases were prepared by standard procedure (22) for filter binding experiments. The GST-topoIIβ fusion proteins were induced with IPTG. The induced and uninduced proteins were fractionated by SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA) with a transfer buffer containing 20 mM Tris (pH 8.0), 137 mM NaCl, 0.2% Tween 20, and incubated with anti-GST mAb or anti-topoIIβ mAb (IGS7) at room temperature without methanol as described previously (21). The membranes were washed with 6 M guanidine hydrochloride, rehydrated, and blocked with 1% dry milk solutions were added at approximately 5 × 10^4 cpm/ml to HBB and incubated overnight. The filters were then washed three times with phosphate-buffered saline (PBS) containing 0.2% Triton X-100 at 4°C, dried, and exposed at −80°C. Positive phases were subsequently isolated and the cDNA inserts were sequenced after subcloning into pBluescript.

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**In Vitro Solution Binding Experiments and Western Blots—**After 5 μg of each GST fusion protein was preabsorbed on the glutathione-Sepharose by incubating at 4°C for 1 h, the nuclear extracts of cells were added to the mixtures and further incubated at 4°C for 2 h. The mixtures were washed three times with PBS-0.1% Nonidet P-40 (PBS-N). The lysates were subjected to SDS-PAGE, and the proteins were transferred to PVDF membranes. The membranes were blocked with Block Ace (Yukijirushi Ltd., Hokkaido, Japan) at room temperature for 1 h, incubated with anti-topoIIβ mAb (IGS7) at 1 h at room temperature, washed three times with a TBS-T buffer (20 mM Tris (pH 7.6), 137 mM NaCl, 0.2% Tween 20), and incubated with peroxidase-conjugated anti-mouse Ig antibody at room temperature for 1 h, washed twice with the lysis buffer. The membranes were developed with an ECL detection system (Amersham).
supernatants were then dialyzed overnight at 4 °C against PBS containing 0.5 mM PMSF. After centrifugation at 15,000 rpm for 20 min, the supernatants were collected and used as the nuclear extracts.

Immunoprecipitation—The cell lysates or the nuclear extracts from 2B4 or DO11.10 T cell hybridoma cells were immunoprecipitated with 5 µg of anti-CD3e mAb 2C11 or anti-human CD3 mAb OKT3 as a control and protein A-Sepharose beads. The precipitates were washed with the lysis buffer containing 0.1–0.5% Nonidet P-40 for the digitonin lysate or with 1% Brij 96-containing buffer for the Brij lysate. Immunoprecipitated proteins were then dialyzed overnight at 4°C against PBS containing 0.1% Brij 96-containing buffer for the Brij lysate. Immunoprecipitates were analyzed on 8% SDS-PAGE.

TCR Stimulation—Stimulation was performed as described previously (24). Briefly, 2B4 cells (4 × 10^5 cells) or murine thymocytes (7 × 10^6 cells) were incubated with 2C11 (10 µg/ml) for 30 min on ice, washed twice with RPMI 1640, and stimulated by adding prewarmed GAH (100 µg/ml) at 37 °C for 2 min. The reaction was stopped by adding ice-cold PBS, and cells were lysed for 30 min at 4 °C in a lysis buffer. Cytosolic extracts and nuclear extracts were prepared according to the same protocol as described above.

Biotinylation on Sorbent—Biotinylation on sorbent was performed as described previously (25). Briefly, cytosolic or nuclear extracts of 2B4 hybridomas (4 × 10^5 cells) and thymocytes (7 × 10^6 cells) were immunoprecipitated with protein A-Sepharose beads coupled with anti-CD3 mAb (HMT3–1) at 4°C for 2 h. The beads were washed three times with the lysis buffer and once with PBS, and proteins on the beads were biotinylated by incubation with 100 µg/ml biotin (Pierce) in 1 ml of the labeling buffer (0.01 M Hepes (pH 8.0), 150 mM NaCl) at 4 °C for 1 h. The beads were washed three times with the lysis buffer, and the proteins were eluted by boiling in the SDS-PAGE sample buffer and were then analyzed by nonreducing-reducing two-dimensional SDS-PAGE (12% for the first dimensional gel under nonreducing condition and 14% for the second dimensional gel under reducing condition).

RESULTS

Cloning of cDNAs Encoding the CD3e-Binding Proteins—A Agt11 expression library prepared from a human T cell line was screened with a probe of 32P-labeled GST fusion protein containing the cytoplasmic domain of the CD3ε chain (GST-ε). Eight positive clones (clones 3, 6, 10, 16, 25, 36, 43, and 46) encoding β-galactosidase fusion proteins were picked up and used for further analysis. Subsequent analysis demonstrated that these (10, 43, 46) and two clones (6, 36) contained overlapping cDNA sequences, respectively. Among the resulting five cDNAs encoding CD3ε-binding proteins, we described the characterization of clone 3 (C3) in the present study.

Topoisomerase I β Specifically Binds to CD3ε—To confirm the binding specificity of C3 to GST-ε, we performed a filter binding assay with lysogenic phase lysates. As shown in Fig. 1, the GST-ε probe bound to a protein of approximately 180 kDa from the IPTG-induced lysate of C3, but not from the uninduced lysate of this clone nor from the induced lysate of an irrelevant clone C1. GST alone as a control did not show any specific interaction. The 180-kDa protein from C3 also interacted with anti-β-galactosidase antibody, confirming that this protein was a β-galactosidase fusion protein (Fig. 1). Sequence analysis showed that C3 contained the C-terminal region of topol β. Topol β has just been cloned recently (18), whereas its isoform, topol α, had been characterized previously. Both isoforms are considered to be involved in DNA replication and transcriptional regulation (26, 27). However, the function of topol β and the functional difference from its isoform are not yet understood. The region of topol β corresponding to C3 was abundant in acidic charged amino acids and potential phosphorylation sites and is considered to be the regulatory region of the enzyme (18).

N Terminus of the Cytoplasmic Region of CD3ε Binds to Topol β—To define the precise binding region of CD3ε to topol β, serial deletion mutants of CD3ε were constructed. The cytoplasmic region was tentatively divided into three regions; the N-terminal region: aa 135–161; the central region, aa 162–169; and the C-terminal region, aa 170–189. The N-terminal region, the central portion, and the C-terminal region contain a basic aa cluster, a proline-rich sequence, and ITAM, respectively (Fig. 2A). We prepared serial deletion constructs by PCR and site-directed mutagenesis in the tyrosine residues of ITAM as shown in Fig. 2A. Each GST fusion protein was induced with IPTG in E. coli and purified on glutathione-Sepharose. Purified proteins were resolved in SDS-PAGE. Coomassie Blue staining showed that all constructs made the expected size of the proteins (Fig. 2B).

The nuclear extracts prepared from 2B4 hybridoma cells were precipitated with each GST-protein prebound on glutathione beads, separated on SDS-PAGE, and transferred to a PVDF membrane. The membrane was blotted with anti-topol β mAb β5A7. GST-ε, -EM3, -EM4, -ED3, -ED5, -ED1, but not GST-ED4 bound to topol β (Fig. 2C). Since GST-ED1 contains only the N-terminal 12 aa, it is likely that the binding region is located in the basic aa cluster in the N-terminal region of CD3ε (Fig. 3A).

We next wanted to determine the specificity of the binding, whether the binding of topol β is specific for CD3ε or whether it also binds to similar signaling molecules such as CD3ζ and FcγR. As shown in Fig. 2C, none of GST-ζ, GST-fyn, or GST alone bound to topol β. In contrast, GST-FcγR was found to bind to topol β (Fig. 2C). Comparing the aa sequences of CD3ε and FcγR, we found that the homologous sequence in the 12 aa binding region of CD3ε was present in FcγR (Fig. 3A). This novel motif contains a basic aa cluster. The CD3ε chain contains clusters of basic aa such as KKKAR and RRR, but it did not bind to topol β, demonstrating that the binding is not due to nonspecific interactions with any clusters of basic aa.

Collectively, these data demonstrate that the binding of CD3ε to topol β is sequence-specific, and only 12 aa of the N-terminus of the cytoplasmic region of CD3ε are sufficient for the interaction.

The Most C-terminal Region of Topol β Binds to CD3ε—Since C3 contained a 1.6-kb fragment corresponding to the C-terminal region of topol β, we employed a filter binding assay to specify the precise binding region to CD3ε. The cDNA fragment was divided into two regions, and the GST fusion constructs were prepared for each region. GST-topol β1 and GST-topol β2 contained the N- and C-terminal halves of C3, respectively (Fig. 4A). Each GST protein was examined for the binding ability to CD3ε. IPTG-induced or uninduced lysate from the bacteria containing each construct was blotted with GST-ε or GST alone. As shown in Fig. 4B, only the induced lysate from GST-topol β2, but not from GST-topol β1, bound specifically to GST-ε. This region of topol β contains a cluster of acidic aa, suggesting that this cluster may be responsible for binding to CD3ε.

CD3ε and CD3ζ Are Present in the Nucleus—Unlike topol α, the expression of topol β is rather restricted, being especially...
high in the thymus (data not shown) (29), suggesting that topoIIβ might be involved in T cell function. Although CD3ε binds specifically to topoIIβ, the obvious question raised was concerning the localization of these two proteins. Whereas CD3ε is a membrane protein, topoIIβ is localized mainly in the nucleus. Therefore, we examined the possibility of whether CD3ε also exists in the nucleus. One piece of evidence which may support this possibility is that both CD3ε and CD3ζ have the consensus sequence corresponding to nuclear localizing signal (NLS). As depicted in Fig. 3B, CD3ε possesses the motif homologous to the NLS of SV40 T antigen (28), and CD3ζ contains the nucleoplasmin-like NLS (bipartite) (29), respectively. Since we were unable to detect CD3ε or CD3ζ in the nuclear fraction by Western blotting (data not shown), a highly sensitive biotinylation method was employed (30) to detect even small amounts of CD3ε or CD3ζ in the nuclear fraction.

To this end, cytosolic and nuclear extracts from thymocytes and 2B4 hybridomas either unstimulated or stimulated by cross-linking with anti-CD3ε mAb were prepared as described under “Materials and Methods.” These extracts were immunoprecipitated with anti-CD3ε mAb HMT3–1 and protein A-Sepharose. Precipitated proteins on the beads were labeled with biotin and analyzed on two-dimensional SDS-PAGE. As shown in Fig. 5, precipitation of the cytosolic fraction showed TCRαβ and CD3ζ dimers as off-diagonal spots and CD3ε as the spots slightly above the diagonal. As expected, both CD3ε and CD3ζ were detected in the nuclear fractions of both plasmic domains of ε, various mutants of ε, ζ, ζc, and FcRγ. Schematic structures of these fusion proteins were shown. B, purified GST fusion proteins were purified on glutathione-Sepharose beads, separated on SDS-PAGE, and stained with Coomassie Blue. The molecular size markers are indicated at the left margin. C, minimum binding region of CD3ε to topoIβ and the specificity of the binding among signaling molecules. The nuclear extracts of T cell hybridomas were precipitated with each GST fusion protein and subjected to SDS-PAGE. Proteins were transferred to membranes, and the membranes were blotted with anti-topoIβ mAb. The arrow indicates topoIβ. The molecular size markers are indicated at the left margin.
thymocytes (Fig. 5, C and D) and 2B4 hybridomas (not shown). The ε chain in the nuclear fraction was observed only upon TCR stimulation, and the amount of CD3ε also increased upon TCR stimulation in thymocytes. CD3ε from the cytosol fraction showed two spots (ε and ε' in Fig. 5B). Since ε' was slightly larger than ε and reacted with anti-phosphotyrosine mAb 4G10 by Western blotting (data not shown), ε' appeared to be phosphorylated ε. On the other hand, most of CD3ε in the nuclear fraction appeared to be the same size as ε', suggesting that most of CD3ε in the nuclear fraction is probably phosphorylated. TCR α and β dimers were barely detected in this fraction, suggesting that the detected CD3ε and CD3ζ in the nuclear fraction were not present as components of the whole TCR-CD3 complex and that the presence of CD3ε and CD3ζ in this fraction did not merely reflect the contamination of the cytosolic fraction.

To confirm the latter issue, the same cytosolic and nuclear fractions used to detect CD3ε were immunoprecipitated and blotted with anti-tubulin mAb, since tubulin is present exclusively in the cytosol (31). As shown in Fig. 6, tubulin was detected only in cytosolic fraction but not at all in the nuclear fraction of our preparations. This result clearly demonstrated that the contamination of the nuclear fraction by cytosol was negligible and that CD3ε is likely to be present in the nucleus.

These results suggest that CD3ε exists in the nucleus especially after TCR activation and that it binds to topolβ in the nucleus in vivo.

Specific Interaction between Topolβ and CD3ε in T Cells—To demonstrate the direct interaction between CD3ε and topolβ in vivo, the nuclear fraction was prepared from DO11.10 hybridoma cells by solubilization with either digitonin or Brij 96 and immunoprecipitated by anti-mouse CD3ε (2C11) mAb and anti-human CD3ε (OKT3) mAb as a control and then analyzed by Western blot with anti-topolβ Ab. As shown in Fig. 7, topolβ was co-precipitated from the nuclear fractions of both digitonin (Fig. 7, lanes 1 and 2)- and Brij 96 (Fig. 7, lanes 3 and 4)-lysed cells. The fact that the association was only observed in digitonin or Brij lysates and that this association was not observed when the immunoprecipitate was washed with the buffer containing a higher concentration of

Fig. 5. CD3ε and CD3ζ are present in the nuclear fraction. 7 × 10⁶ thymocytes, unstimulated (A, C) or stimulated with anti-CD3ε mAb 2C11 (B, D), were lysed, and the cytosol fractions (A, B) and the nuclear fractions (C, D) were prepared as described under "Materials and Methods." Each fraction was immunoprecipitated with HMT3-1 coupled with protein A-Sepharose beads. Precipitated proteins were biotinylated on the beads and subjected to two-dimensional nonreducing (12%)/reducing (14%) SDS-PAGE. The proteins were transferred onto membranes and developed with an ECL detection system. The spots corresponding to CD3ε (ε), possibly phosphorylated ε (ε'), and CD3ζ homodimers (ζ) were indicated.

Fig. 6. Negligible carryover of the cytosol fraction into the nuclear fraction of the thymocyte preparation. The total lysates in the same cytosol (C) and nuclear fractions (N) described in the legend to Fig. 5 were subjected to 14% SDS-PAGE, and the proteins were transferred to membranes. The membrane was stained with Coomassie Blue (A) or blotted with anti-tubulin mAb followed by development with an ECL detection system (B). The arrow indicates tubulin. The molecular size markers are indicated at the left margin.
Nonidet P-40 than 0.3% suggested that the interaction between topol β and CD3ε was not strong in vivo (data not shown).

**DISCUSSION**

We have cloned topol β as a CD3ε-binding molecule by western screening procedure. We determined the binding regions of both CD3ε and topol β as well as the specificity of the interaction. The binding region of CD3ε to topol β was localized within 12 aa in the N-terminal region containing a novel motif of basic aa cluster. A similar motif was also found in FcRγ. Since ITAM has been thought to be the only functional domain within CD3ε, this is the first report that a specific binding site other than ITAM exists in the cytoplasmic region of CD3ε. The binding region was composed of a novel motif of a basic aa cluster. Although CD3ε has other basic aa clusters, ζ did not bind to topol β, confirming the specific binding of topol β to CD3ε. It has been suggested that the TCR-CD3 complex is composed of two distinct activation modules (12, 13). Distinct signals are transduced through CD3ε and CD3ζ. The binding of ε, but not ζ, to topol β may represent one of such differences in signal transduction.

In terms of the physiological interaction of these two proteins, the question of their localization then arises. Topol β is considered to be located in the nucleus whereas CD3ε is a membrane protein, and this question was therefore answered by demonstrating the translocation of CD3ε into the nucleus. Our finding that CD3ε and CD3ζ have NLS-like sequences in their cytoplasmic region supports the idea of translocation of a part of CD3ε and CD3ζ into the nucleus. There have been several reports about transmembrane-type receptors such as epidermal growth factor receptor and platelet-derived growth factor receptor, similar to CD3ε, translocating to the nucleus (32). Luton et al. (33) reported that the TCR-CD3 complex translocated to the cytoskeleton-associated insoluble fraction upon TCR stimulation. Considering that the nuclear fraction was involved in the insoluble fraction and nuclear translocation was linked with cytoskeleton-associated proteins such as actin filament, their observation may partly reflect the translocation of the CD3 chains to the nucleus. Although we failed to detect CD3ε or CD3ζ in the nuclear fraction by Western blotting, we eventually succeeded in detecting both CD3ε and CD3ζ in the nuclear fractions of normal thymocytes and T hybridoma cells by labeling with a sensitive biotinylation method. Importantly, the amounts of CD3ε and CD3ζ were increased upon TCR activation while those in the cytosolic fraction did not seem to be changed. Taken together with the result that our preparation of the nuclear fraction did not contain any detectable contamination by the cytosolic fraction, we demonstrated for the first time that CD3ε and/or exist in the nucleus in normal T cells and increased upon T cell activation. By immunoprecipitation with anti-CD3ε mAb, we clearly demonstrated the direct in vivo association between CD3ε and topol β in the nuclear fraction of T cells. These data indicate that CD3ε is translocated into the nucleus upon T cell activation and interacts with topol β.

In addition, there is a possibility that CD3ε may also bind to topol β present in cytosol. Indeed, it has recently been observed that topol β is transiently distributed to the cytoplasm during the mitotic stage, whereas topol α is associated tightly with chromosomes constantly throughout the cell cycle.

Previous analyses of T cells expressing extensive deletion constructs of CD3ε showed that ITAM is both necessary and sufficient for IL-2 production (11). These results indicate that the topol β-binding motif of CD3ε is not prerequisite for IL-2 production. However, it has recently been reported that inhibitors of topol I, quinolon derivatives, up-regulated IL-2 production upon TCR stimulation (34). Since the CD3ε-binding site is the regulatory region of topol β in vitro, which was also suggested by the fact that an anti-topol β mAb specific for the C terminus region of topol β inhibited the enzymatic activity, the binding of CD3ε may also block the enzymatic function and result in the super induction of IL-2 production, similar to the treatment of T cells with the topol I inhibitors. Moreover, topol I inhibitors are known to induce apoptosis (35, 36). Growth arrest and subsequent apoptosis is induced in T cells upon TCR stimulation. The binding of CD3ε to the regulatory region of topol I might modulate the function similarly to the inhibitors in vivo. Although we tested this hypothesis by performing a decatenation assay with nuclear extracts, we failed to detect any significant effect of the GST-e binding on the in vitro function of topol β (data not shown). We assumed that the failure of modulation in the overall decatenation assay may be due to the dominant function of topoII even under the condition that CD3ε binding may modify the function of topol β protein. Analysis by the use of recombinant topol β, which is not available yet, will be required.

Collectively, CD3ε appears to possess two distinct functional domains; whereas ITAM is phosphorylated and stimulates ZAP70 and the following activation pathway in the cytoplasm, the N-terminal motif of CD3ε found in this study plays a functional role in the nucleus after translocation.

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