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Immunology

Expression of T3 in association with a molecule distinct from the T-cell antigen receptor heterodimer

(coprecipitation/T3–Ti complex/T-cell antigen receptor γ-chain/T-cell subpopulation/Tp55-60)

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ABSTRACT **The T-cell antigen receptor consists of a disulfide-linked heterodimer (Ti) that is associated with another set of three nonpolymorphic, noncovalently linked peptides termed "T3." The cell surface expression of T3 has been thought to depend upon association with Ti. In this study, we demonstrate that T3 can be expressed in the absence of an associated Ti molecule on a T-cell leukemic line, PEER. Instead, on this cell line, T3 appears to be expressed in association with a 55- to 60-kDa glycoprotein that has a peptide backbone of 29 kDa. PEER fails to express Ti α-chain transcripts but does express Ti β- and γ-chain transcripts. Using a monoclonal antibody that reacts with nonpolymorphic epitopes expressed on Ti, WT31, we demonstrate that PEER fails to react with this antibody but does react with three independently derived anti-T3 antibodies. Moreover, a small subpopulation of T3-positive peripheral blood lymphocytes, like PEER, fails to express the antigenic determinants recognized by WT31. These results suggest that, on these normal lymphocytes, T3 may likewise be associated with a non-Ti molecule. The possibility that the 55- to 60-kDa molecule expressed on PEER, termed "Tp55–60," represents the protein product of the previously identified Ti γ-chain gene is discussed.**

Monoclonal antibodies (mAb) reactive with unique clone-specific antigenic epitopes were used to first identify the T-cell antigen receptor which consists of a heterodimer, Ti, associated with a set of three peptides, T3 (1–3). On murine and human cells, Ti is an 80- to 90-kDa disulfide-linked heterodimer composed of an acidic α-chain and a more basic β-chain. Both chains are glycosylated and contain variable and constant region domains (4–6). The genes encoding these chains have been identified and are preferentially expressed in T cells; but, at the nucleotide level, they exhibit substantial homology to the immunoglobulin genes (7–11). Studies of the genomic organization of these genes have revealed that, like immunoglobulin genes, rearrangement of gene elements accounts for the generation of antigen receptor molecules with differing specificities (12). In the course of studies that were aimed at identifying the α-chain cDNA in the mouse, another gene that undergoes rearrangement and is preferentially expressed in T cells was identified (13). This gene has been termed the Ti γ-chain gene and a homologous gene has been identified in the human (14). However, to date no protein product of this gene has been identified.

On human T cells, the Ti heterodimer is noncovalently associated with an antigen complex termed T3. T3 consists of at least three nonpolymorphic, noncovalently associated peptides (16). Thus, in the human, T3 consists of a nonglycosylated 21-kDa chain (T3 ε chain) and glycosylated 22-kDa and 26-kDa chains (T3 δ and γ chains, respectively). Homologous proteins have been identified in the mouse, but in addition to these three chains, another 32-kDa disulfide-linked homodimer has been observed (17, 18). The cDNA encoding the T3 δ chain has been isolated (20). Whereas the function of T3 is not clear, it seems likely that it may be involved in transmembrane signaling events. Hence, mAbs against either Ti or T3 can function as agonists in the activation of T cells (21–23). Early events associated with the perturbation of either Ti or T3 are the hydrolysis of phosphatidylinositol bisphosphate and the consequent increase in cytoplasmic free calcium (24–27).

The nature of the physical association between T3 and Ti is not clear. A variety of methods have been used to demonstrate their association: (*i*) coprecipitation of T3 and Ti from lysates of iodinated cell surface proteins (28, 29); (*ii*) comodulation of T3 and Ti from the surface of T cells exposed to either anti-Ti or anti-T3 mAb (2); and (*iii*) chemical-crosslinking of T3 and Ti with bifunctional reagents (17, 30). Sequence data demonstrating an intramembranous aspartic acid residue in the T3 δ chain and highly conserved lysine residues in the human Ti α- and β-chain transmembrane regions has led to the speculation that these oppositely charged residues may be involved in the association of T3 and Ti (7–11, 20). Cell surface expression of T3 depends upon a concomitant expression of Ti in at least one human T-cell line, Jurkat (23). Mutants of Jurkat that failed to express both T3 and Ti on the cell surface were found to contain intracellular T3, but some of these mutants failed to express full-length β-chain transcripts (31). Cell surface expression of both T3 and Ti could be reconstituted in one such mutant by transfection of the Ti β-chain cDNA (31). Thus, the association of T3 with Ti would appear to be obligatory for cell surface expression of T3. In the current study, we demonstrate that T3 is expressed on a human T-cell line in the absence of a Ti α/β-chain heterodimer. Instead, the expressed T3 appears to be associated with a previously undescribed 55- to 60-kDa molecule. Furthermore, a subpopulation of T3-positive peripheral blood lymphocytes (PBL) that also appear to lack cell surface Ti is identified and may be the population of origin of the cell line described.

MATERIALS AND METHODS

Cells. E6-1, a subclone of Jurkat-FHCRC, has been described and maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, antibiotics, and glutamine (23). PEER and HPB-ALL were obtained from Richard Miller and maintained in the above medium (15, 19). Peripheral blood mononuclear cells were obtained from healthy volunteers and isolated by Ficoll/Hypaque density gradient centrifugation. Human thymus was obtained from pediatric cardiac

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Abbreviations: mAb, monoclonal antibody; Endo F, endoglycosidase F; PBL, peripheral blood lymphocytes; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.

surgery specimens, and a cell suspension was prepared, followed by Ficoll/Hypaque density gradient centrifugation.

Antibodies. The following ascitic fluids containing anti-T3 antibodies were obtained from previously described sources: anti-Leu-4, 64.1, and OKT3 (24). C305 is a mAb produced in this laboratory against the Ti heterodimer expressed on Jurkat cells (23). WT31 ascites and fluorescein isothiocyanate-coupled WT31 were obtained from Patrick Kung. Biotin- and phycoerythrin-conjugated anti-Leu-4 or streptavidin were obtained from Becton Dickinson. Rabbit anti-mouse IgG serum was produced in this laboratory.

Immunoprecipitation. Cells (2×10^7 per 0.5 ml of phosphate-buffered saline) were radioiodinated with 1 mCi (1 Ci = 37 GBq) of ^{125}I (Amersham) as described (23). The cells were solubilized in lysis buffer containing 10 mM Tris-buffered saline (pH 7.8), 10 mM iodoacetamide (Sigma), 0.01% sodium azide, protease inhibitors, and the indicated concentration of the detergent used as described (30). Lysates were precleared on formalin-fixed staphylococci, Cowan strain A (Calbiochem), and iodinated cell surface proteins were isolated by using preformed immunocomplexes as described (31). Immunoprecipitates were washed in the same detergent used in the lysis procedure with sucrose gradients (15). The washed precipitates were dissolved in $NaDodSO_4$ sample buffer and analyzed by discontinous $NaDodSO_4$/PAGE as described (23). Endoglycosidase F (Endo F) treatment of washed immunoprecipitates was performed in 100 mM sodium phosphate, pH 6.1/50 mM EDTA/0.1% $NaDodSO_4$ at 37°C for 18 hr with 8 units of Endo F (New England Nuclear) per immunoprecipitate.

Blot-Hybridization Analysis. Whole cellular RNA was isolated by using guanidine thiocyanate and centrifugation over a cesium chloride cushion (32). RNA (30 μg per lane) was electrophoresed in 1% agarose gels containing formaldehyde and blotted onto nylon membranes (Micron Separations, Honeoye Falls, NY). After UV crosslinking, blots were prehybridized in 1 M NaCl/1% $NaDodSO_4$ and then hybridized with a ^{32}P-labeled denatured cDNA probe (5×10^5 cpm/ml; (10^8 cpm/μg of DNA) at 65°C for 18 hr in 1% $NaDodSO_4$/1 M NaCl/100 μg of denatured salmon sperm DNA per ml. Blots were washed as described (31). Probes were labeled by the random primer method as described (33). Probes used were: (*i*) the *Eco*RI insert of pTi-α (Ti α chain) provided by T. W. Mak (10); (*ii*) the 2.2-kb *Xba* I–*Bam*HI fragment of pTBF-neo (Ti β chain) (31); (*iii*) the *Eco*RI insert of Fγ7 (Ti γ chain) provided by D. Littman (the constant region sequence of this cDNA is identical to that previously reported for the human Ti γ chain; D. Littman, personal communication); and (*iv*) the *Pst* I–*Sal* I insert of pPGBC9 (T3 δ chain) kindly provided by P. Van den Elsen and C. Terhorst (20).

Immunofluorescence. Direct or indirect immunofluorescence was performed by standard methods as described (23). One- and two-color analysis was performed on the FACS IV cell sorter (Becton Dickinson). Approximately 30,000 events were collected per analysis.

RESULTS

Coprecipitation of T3 and Ti Depends upon Detergent Concentration. Previous studies have not been uniformly successful in demonstrating an association between T3 and Ti in immunoprecipitation studies (1–3, 17, 23, 29). Further understanding of their association might be gained by understanding the conditions required for their coprecipitation. Varying salt concentrations did not facilitate the coprecipitation of T3 and Ti (data not shown). Studies of the IgE Fc receptor have suggested that association between various components of that molecular complex are dependent upon protein–phospholipid interactions, which are disrupted by excess detergent (34). When a variety of detergent systems, including Nonidet P-40, Triton X-100, and CHAPS {3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate}, was used, the association between T3 and Ti could be preserved if cells were lysed with a detergent concentration near its critical micelle concentration. An example of one such experiment is shown in Fig. 1*A*. When radioiodinated Jurkat cells were lysed in CHAPS and immunoprecipitated with anti-Leu-4 mAb (anti-T3), only T3 proteins were isolated with 10 mM CHAPS. With 5 mM CHAPS (the critical micelle concentration in this buffer), however, Ti was coprecipitated with T3, and some T3 could be seen in immunoprecipitates prepared with C305 (anti-Ti) at this same detergent concentration. Solubilization did not occur at lower detergent concentrations. By using this technique, an 80- to 90-kDa disulfide-linked heterodimer was coprecipitated with T3 from a variety of T cells, including thymocytes, peripheral T cells, and the human T-cell lines Jurkat, HPB-ALL, and HuT 78 (data not shown).

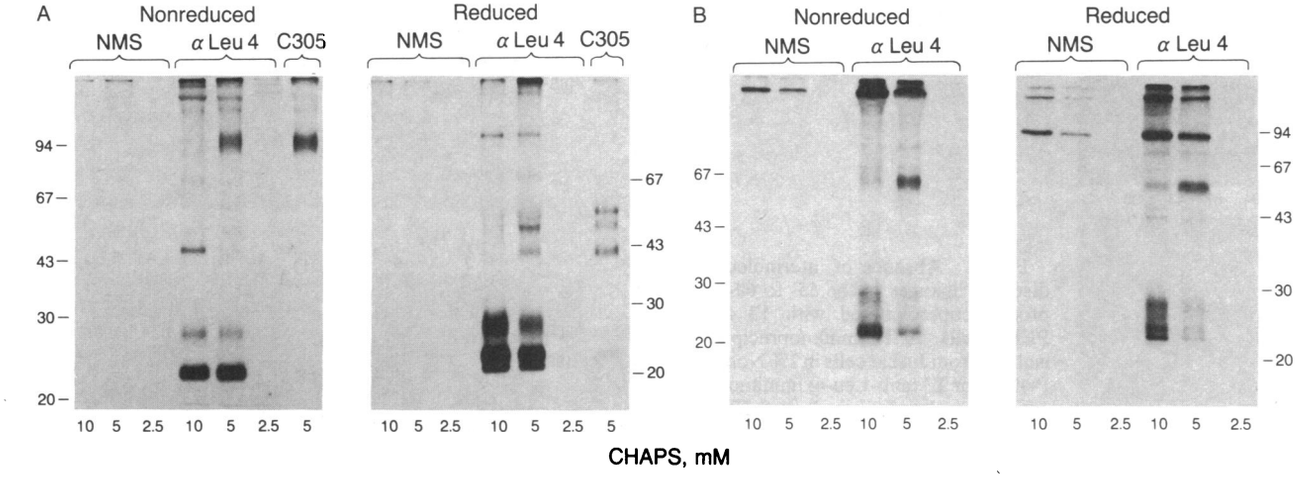

FIG. 1. Coprecipitation of T3 with Ti from Jurkat cells (*A*) or with a 55- to 60-kDa molecule from PEER cells (*B*). Radioiodinated cells were solubilized in lysis buffer containing the indicated concentration of CHAPS detergent. Immunoprecipitates were isolated with the indicated antibodies (NMS, normal mouse serum; α Leu 4, anti-Leu-4, and C305, anti-Ti of Jurkat) and analyzed by $NaDodSO_4$/PAGE with 11% gels. The relative mobilities of molecular mass standards in kDa are given in the margins. Note that the 46-kDa band seen in the T3 immunoprecipitate isolated from Jurkat cells solubilized in 10 mM CHAPS analyzed under nonreducing conditions has not been reproducibly observed and may result from *in vitro* formation of intermolecular disulfide bonds between T3 components.

Coprecipitation of T3 with a 55- to 60-kDa Molecule on PEER. During the course of our studies, we attempted to coprecipitate the Ti molecule from the T3-positive human T-cell line PEER. However, using the CHAPS detergent as described above, we failed to detect the expected iodinated 80- to 90-kDa disulfide-linked heterodimer in T3 immunoprecipitates of PEER. Instead, we routinely have coprecipitated a 55- to 60-kDa protein when using CHAPS at 5 mM (Fig. 1*B*). The mobility of this protein did not substantially change with reduction of disulfide bonds by treatment with 2-mercaptoethanol (Fig. 1*B*). Further evidence that this protein did not contain interchain disulfide bonds is presented in Fig. 2, in which immunoprecipitates of Ti isolated from Jurkat (Fig. 2*A*) or the T3–Ti complexes isolated with anti-Leu-4 from Jurkat (Fig. 2*B*) or PEER (Fig. 2*C*) cells in 5 mM CHAPS were electrophoresed under nonreducing conditions in the first dimension, followed by reduction with 2-mercaptoethanol and electrophoresis in the second dimension. Clearly, the 55- to 60-kDa band that coprecipitates with T3 from PEER does not contain intermolecular disulfide bonds. The position of this band on the diagonal would suggest the absence of intrachain disulfide bonds but does not exclude the possibility of such linkages. The positions of the lower molecular weight bands (20–26 kDa) representing T3 on PEER correlate well with the positions of T3 chains isolated from Jurkat cells. These results suggest that T3 is expressed on the plasma membrane of PEER in association with a molecule other than Ti. To further characterize the 55- to 60-kDa band associated with T3 in the PEER lysates, the presence of N-linked carbohydrate side chains was examined. T3 immunoprecipitates of PEER prepared in 5 mM CHAPS were subjected to Endo F digestion. This resulted in the appearance of a 29-kDa band, presumably derived from the 55- to 60-kDa band, as well as the 14- and 16-kDa bands anticipated from Endo F digestion of T3 (Fig. 3) (16). These results suggest the presence of multiple N-linked glycosylation sites and a possible peptide backbone of 29 kDa for the 55- to 60-kDa protein of interest.

Absence of Ti α-Chain Transcripts in PEER. To further explore the possibility that T3 is expressed in the absence of a Ti α/β-chain heterodimer, blot-hybridizations of whole cellular RNA were prepared from PEER and several other T-cell lines. When these blots were hybridized with a Ti α-chain probe, no Ti α-chain transcripts could be detected in PEER, in marked contrast to the other T-cell lines examined (Fig. 4*A*, lane 4). PEER did contain low levels of Ti β-chain transcripts (Fig. 4*B*, lane 4) and abundant levels of Ti γ-chain (Fig. 4*C*, lane 4) and T3 δ-chain transcripts (Fig. 4*D*, lane 4).

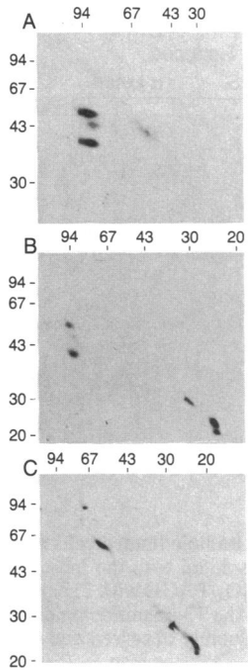

FIG. 2. Absence of intermolecular disulfide linkages in the 55- to 60-kDa protein coprecipitated with T3 from PEER cells. A Ti immunoprecipitate isolated from Jurkat cells in 1% Nonidet P-40 (*A*) or T3 (anti-Leu-4) immunoprecipitates isolated from Jurkat (*B*) or PEER (*C*) cells in 5 mM CHAPS were electrophoresed under nonreducing conditions in the first dimension (from left to right) followed by reduction in 2-mercaptoethanol and electrophoresis in the second dimension (from top to bottom). Mobilities of molecular mass standards in kDa are indicated in the margins.

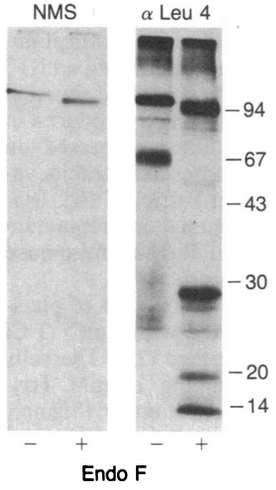

FIG. 3. Endo F treatment of the 55- to 60-kDa molecule coprecipitated from PEER cells reveals a 29-kDa peptide backbone. Control normal mouse serum (NMS) or T3 [anti (α)-Leu-4] immunoprecipitates isolated from PEER cells in 5 mM CHAPS were incubated in buffer only or in 8 units of Endo F for 18 hr at 37°C, followed by analysis with $NaDodSO_4$/PAGE as indicated.

Lack of Reactivity of PEER with an Anti-Ti mAb. WT31, a mAb reactive with most peripheral T cells, was initially felt to react with T3 based on its mitogenic activity (35). However, subsequent immunoprecipitation studies revealed that WT31 interacts with a nonpolymorphic determinant expressed on Ti (29). Therefore, it was of interest to examine the ability of WT31 to react with PEER. Immunofluorescent studies were performed and analyzed by simultaneous two-color fluorescence flow cytometry, staining Jurkat, PEER, peripheral blood mononuclear leukocytes and thymocytes with anti-T3 (anti-Leu-4) and WT31. In order to accomplish this we used WT31 conjugated to fluorescein and anti-Leu-4 conjugated with biotin together with streptavidin conjugated with phycoerythrin.

When Jurkat cells were thus analyzed, a relatively good correlation existed between the intensity of staining for T3 and Ti epitopes. Thus, the diagonal distribution of stained cells depicted on the contour histogram of Fig. 5*A* suggests

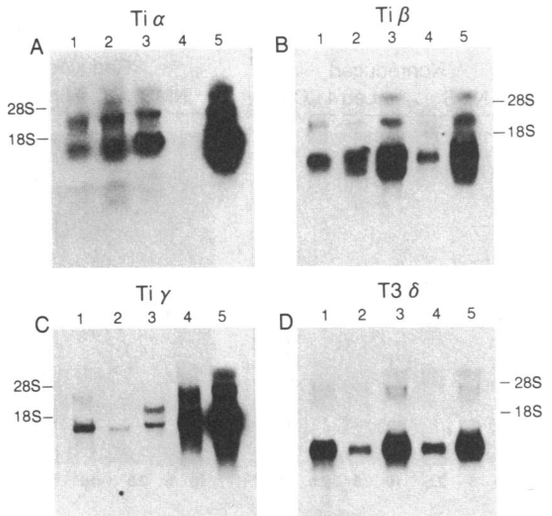

FIG. 4. Failure to detect Ti α-chain transcripts in PEER cells. Blots were prepared with 30 μg of whole cellular RNA isolated from cell lines CEM (lane 1), HuT 78 (lane 2), Jurkat (lane 3), PEER (lane 4), and HPB-ALL (lane 5) and hybridized with the indicated ^{32}P-labeled cDNA probes described in *Materials and Methods*. The positions of 28S and 18S ribosomal RNAs are indicated.

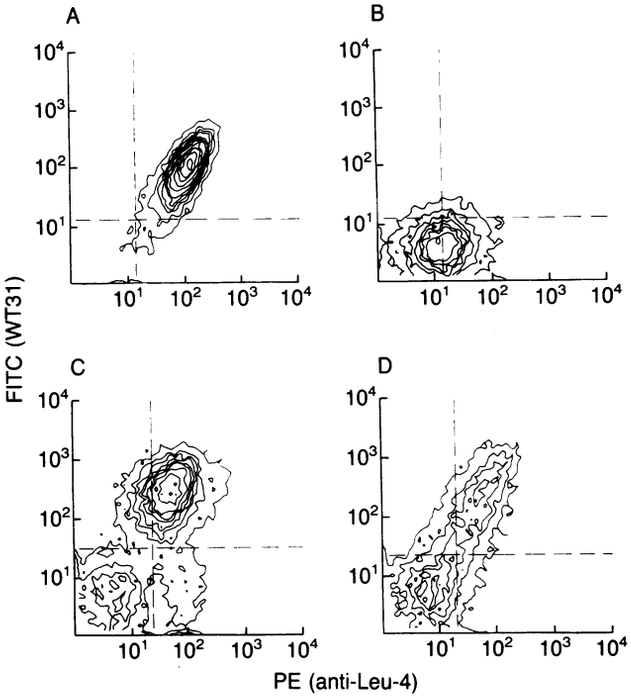

FIG. 5. Lack of reactivity of PEER and a subpopulation of T3-positive PBL with WT31. Simultaneous two-color immunofluorescent analysis was performed by flow cytometry of Jurkat (*A*), PEER (*B*), PBL (*C*), and thymocyte (*D*) cell populations using fluorescein isothiocyanate (FITC)-conjugated WT31 (vertical axis) and biotin-conjugated anti-Leu-4 plus phycoerythrin (PE)-conjugated streptavidin (horizontal axis). Relative fluorescein vs. phycoerythrin fluorescence of the indicated cell populations is depicted by the contour histograms on relative logarithmic scales. More than 97% of the analyzed cells that were stained with control nonreactive antibodies were contained in the area enclosed by the dashed lines in the lower left corner of each histogram.

a stoichiometric relationship between determinants detected by the anti-T3 (anti-Leu-4) and WT31 mAbs. This is in keeping with the notion that T3 and Ti antigenic determinants are expressed on the same molecular complex. In contrast, Fig. 5*B* demonstrates that WT31 failed to react appreciably with PEER, but T3 determinants were detected with anti-Leu-4. This observation has been consistently observed in five separate experiments using one- or two-color immunofluorescence and three independently derived anti-T3 mAbs (anti-Leu-4, OKT3, and 64.1) and WT31. Furthermore, we have failed to immunoprecipitate any molecule from radioiodinated lysates of PEER with WT31 in contrast to the 90-kDa heterodimer immunoprecipitated from Jurkat lysates with WT31 (data not shown).

T3-Positive PBL That Do Not Express Ti. Since PEER appeared to represent an unusual example of a cell line that expressed T3 in association with a non-Ti molecule, it was of interest to determine whether it might be representative of an analogous cell population in normal PBL or thymus. The distinct pattern of reactivity of PEER cells with anti-T3 and WT31, as compared to Jurkat, suggested that these antibodies could be used to search for such cells by screening for a similar reactivity pattern. When PBL were examined, an expected T3-negative/WT31-negative fraction of cells, representing the B-cell population (≈25%), was observed (Fig. 5*C*). Interestingly, two populations of T3-positive PBL were detected. The major T3-positive population (≈65% of the total lymphocytes and 92% of T3-positive cells) was also WT31-positive and, thus, had a staining pattern similar to that observed with Jurkat. On the other hand, a second minor T3-positive population (between 0.5% and 8% of the lymphocytes in five different individuals) failed to react with WT31, resembling the profile expressed by PEER (Fig. 5*C*). These results suggest that T3 may not be associated with an α/β-chain heterodimer on a small fraction of PBL. When human thymocytes were examined, no distinct T3-positive/WT31-negative population could be detected (Fig. 5*D*). Rather, a continuum of T3-negative/WT31-negative to T3-positive/WT31-positive cells were observed.

DISCUSSION

Previous studies had demonstrated that T3 was noncovalently associated with the antigen receptor heterodimer Ti (2, 17, 28–30). This association was shown to be necessary for cell surface expression of T3 on a human T-cell leukemic line (23, 31). In the present study, three lines of evidence were presented that suggest that T3 on the human T-cell line PEER is associated with a molecule that is distinct from the α/β-chain heterodimer comprising Ti: (*i*) rather than an 80- to 90-kDa disulfide-linked heterodimer, a cell surface 55- to 60-kDa glycoprotein was coimmunoprecipitated with T3 from PEER; (*ii*) blot-hybridization analysis failed to reveal Ti α-chain transcripts in PEER; (*iii*) in contrast to other cells that expressed T3 in association with Ti, PEER reacted poorly with the anti-Ti constant region mAb WT31. Moreover, in these studies, we describe a population of T3-positive PBL that appear to lack Ti molecules detected by mAb WT31. These cells may represent the normal population of cells from which PEER has arisen.

The nature of the association between T3 and Ti has been unclear. These studies were initially undertaken to gain an understanding of their interactions. In previous studies, Ti was variably coimmunoprecipitated with T3 (2, 4, 18, 28, 29). In the present studies, coprecipitation of T3 and Ti could be routinely observed with multiple T3-positive cell populations if cell surface proteins were solubilized at limiting detergent concentrations. This technology was based on previous studies of the IgE Fc receptor on mast cells in which exogenous phospholipid or limiting concentrations of detergent were used to enable the coisolation of Fc receptor-associated proteins (34). Those studies suggested protein–phospholipid interactions were essential in preserving the integrity of the IgE Fc receptor molecular complex during isolation. Thus, similar hydrophobic protein–phospholipid associations appear to play a role in the case of the T3 and Ti association as well as the 55- to 60-kDa glycoprotein associated with T3 on PEER.

Based on this technology, we identified a 55- to 60-kDa glycoprotein distinct from Ti, hereafter termed Tp55–60, which appears to be associated with T3 on the cell line PEER. This glycoprotein lacks interchain disulfide linkages and appears to consist of a single glycosylated chain, though the presence of a noncovalently associated second component (which could be poorly radioiodinated) cannot be excluded. The stoichiometry of T3 and Tp55 is not clear from these studies. Thus, Tp55–60 could also exist as a noncovalently linked homodimer. Based on Endo F digestion, Tp55–60 has abundant N-linked carbohydrate side chains and has a peptide core of ≈29 kDa. The size of the peptide backbone is in keeping with the general size of the Ti β and α peptide chains (7–10). However, glycosylated Tp55–60 is considerably larger than any reported human Ti α or β chains (2, 5, 15, 23). Moreover, PEER contains no Ti α-chain transcripts. We have examined the possibility that Tp55-60 represents one of the previously reported T-cell surface proteins with similar molecular masses; namely, T4, T11, or the interleukin 2 receptor. However, PEER fails to express any of these three antigens by immunofluorescence or immunoprecipitation (data not presented). In fact, the only other T cell-specific surface antigens we have detected on PEER are T1, a 67-kDa protein, and Tp44, an 80- to 90-kDa homodimer,

which do not comodulate with T3 on PEER and are substantially larger than Tp55–60 in immunoprecipitation studies (data not presented) (36). Thus, Tp55–60 does not appear to have been previously described. At this point, however, it is tempting to speculate that Tp55–60 may represent the as yet unidentified protein product of the Ti γ-chain gene. The Ti γ-chain gene undergoes rearrangement, it is T cell-specific, and its product exhibits some sequence homology to the Ti α- and β-genes (13, 14). Thus, it has been thought that the Ti γ-chain gene likely encodes a T-cell surface recognition molecule. Like the α and β chains, the Ti γ chain also has a conserved lysine residue in the transmembrane region (13). Therefore, it is quite possible that the protein product of the Ti γ-chain gene would be expected to be associated with T3 on the cell surface. The predicted molecular weight of the γ-chain peptide would generally be consistent with the peptide backbone of Tp55–60. PEER contains abundant Ti γ-chain transcripts. Although the Ti γ chain first isolated in the mouse lacks N-linked glycosylation sites, the Ti γ-chain gene isolated in the human could contain N-linked carbohydrate side chains (13, 37) and, in a recently reported study, could contain as many as five N-linked glycosylation sites (38). Based on the conserved cysteines described in the three Ti chains, one might have anticipated that the Ti γ-chain protein product would be disulfide-linked to another chain. However, a C_γ gene, recently isolated, lacks a cysteine residue and, hence, could be the C_γ expressed in PEER (38). The cDNA sequences of previously reported Ti γ chains would have also predicted the presence of intrachain disulfide bonds (13, 37, 38). If Tp55–60 contained intrachain disulfide linkages, it might have been expected to migrate above the diagonal in Fig. 2*C*. However, because of the high degree of glycosylation of this polypeptide, Tp55–60 may have behaved anomalously under these circumstances. Given the high level of γ-chain transcripts in the thymus, the detection of a T3-positive/WT31-negative cell population in the thymus would be anticipated (39). The failure to detect such a population may be due to the insensitivity of the technology used. Definitive proof that Tp55–60 represents the Ti γ-chain gene product will depend upon sequence studies.

These studies appear to define a unique set of T cells that express T3 in the absence of the T-cell antigen receptor antigenic determinants, as detected by WT31. PEER may represent an immortalized example of this subset. Initially isolated from a patient with an acute lymphocytic leukemia with pronounced lymphadenopathy, PEER has the following phenotype: $T1^+$,$T3^+$,$T4^-$,$T6^-$,$T8^-$,$T11^-$,HLA-DR^-,$Tp44^+$, and interleukin 2 $receptor^-$ (15). Thus, it does not clearly fit one of the previously described subpopulations of T cells. The PBL that express T3 but not determinants detected by WT31 have not been fully characterized; thus, their phenotype is not necessarily identical to that of PEER. One cannot exclude the possibility that they might express a Ti α/β-chain heterodimer not detected by WT31. Due to their small numbers, the biochemical characterization of a possible T3-associated protein on these PBL has not yet been accomplished, but it is tempting to speculate that it will prove to be a 55- to 60-kDa molecule. The function of these cells and the specificity of their receptors will provide areas for further study.

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