Activation-induced Modification in the CD3 Complex of the γδ T Cell Receptor

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Abstract

The T cell antigen receptor complexes expressed on αβ and γδ T cells differ not only in their respective clonotypic heterodimers but also in the subunit composition of their CD3 complexes. The γδ T cell receptors (TCRs) expressed on ex vivo γδ T cells lack CD3δ, whereas αβ TCRs contain CD3δ. While this result correlates with the phenotype of CD3δ$^{-/-}$ mice, in which γδ T cell development is unaffected, it is inconsistent with the results of previous studies reporting that CD3δ is a component of the γδ TCR. Since earlier studies examined the subunit composition of γδ TCRs expressed on activated and expanded peripheral γδ T cells or γδ TCR^{+} intestinal intraepithelial lymphocytes, we hypothesized that activation and expansion may lead to changes in the CD3 subunit composition of the γδ TCR. Here, we report that activation and expansion do in fact result in the inclusion of a protein, comparable in mass and mobility to CD3δ, in the γδ TCR. Further analyses revealed that this protein is not CD3δ, but instead is a differentially glycosylated form of CD3γ. These results provide further evidence for a major difference in the subunit composition of αβ- and γδ TCR complexes and raise the possibility that modification of CD3γ may have important functional consequences in activated γδ T cells.

Key words: T cell receptor • structure • glycosylation • CD3 • activation

Introduction

The antigen receptor on T cells is a multisubunit complex composed of a clonotypic heterodimer and four invariant signaling subunits, CD3γ, CD3δ, CD3ε, and TCR-ζ. The generally accepted stoichiometry of the αβ TCR is non-γδ TCR-αβ, CD3γε, CD3δε, and TCR-ζζ (1). Previous data obtained from expanded populations of peripheral γδ T cells suggested a similar structure for the γδ TCR, except that the γδ TCR could contain the TCR-ζ family member, FcεR1 γ (FcRγ), as a homodimer or as a heterodimer with TCR-ζ in lieu of the TCR-ζ homodimer (2). However, recent data have called for a revision in this model of γδ TCR structure. For example, the observation that FcRγ is a component of the γδ TCR conflicts with the findings that FcRγ transcripts are not detected in purified thymic and peripheral γδ T cells (3) and that γδ T cell development is unaffected in FcRγ$^{-/-}$ mice (4, 5). We extended these studies by analyzing the subunit composition of γδ TCR complexes on ex vivo γδ T cells from γδ TCR Tg and nonγδ-TCR Tg mice (6). Biochemical analyses showed that TCR-ζ is a component of the γδ TCR on ex vivo γδ T cells, while FcRγ is not. However, after in vitro activation and expansion, FcRγ is expressed and incorporated into the γδ TCR complex (6). These data show that activation and expansion induce changes in the γδ TCR complex and demonstrate that the subunit composition of γδ TCRs expressed by activated and expanded populations of γδ T cells does not accurately represent the subunit composition of TCR complexes expressed by primary γδ T cells.

A more striking inconsistency with the previous model of γδ TCR structure was the finding that the γδ TCRs expressed on ex vivo thymic and lymph node γδ T cells do not contain CD3δ (6). This result appears to contradict previous studies, in which the CD3 subunit composition of the αβ TCR and γδ TCR was found to be identical (2, 7,

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8). As these previous studies used expanded populations of peripheral γδ T cells, γδ TCR$^+$ intestinal intraepithelial lymphocytes (iIELs)* and γδ T cell hybridomas as sources of γδ T cells, it is possible that activation and expansion induced a change in the subunit composition of the CD3 complex. To test this, we compared the subunit composition of TCR complexes expressed by γδ T cells before and after in vitro activation and expansion. Here, we report the detection of a protein, equivalent in mass to CD3δ, in the γδ TCR after activation and expansion. Biochemical analyses revealed that this protein was not CD3δ but a differentially glycosylated form of CD3γ. In addition, modification of the CD3γ subunit was observed in in vitro–activated αβ T cells and in iIELs.

Together, these results demonstrate that the protein initially thought to be CD3δ in the γδ TCR complex is, instead, a differentially glycosylated form of CD3γ and provide further substantiation for the conclusion that the αβ TCR and γδ TCR complexes differ in their subunit composition.

Materials and Methods

Mice. B6.129-TCR-β$^{-/-}$ (TCR-β$^{-/-}$) mice (9) were purchased from The Jackson Laboratory. C57BL/6 (B6), C57BL/6-V_γ6-J_γ1-C_γ1/V_δ1-D_δ2-J_δ2-C_δ γδ TCR Tg (γδ TCR Tg, line 134) (10), and C57BL/6-CD3δ$^{-/-}$ (CD3δ$^{-/-}$) (11) mice were maintained in our animal facility. All experimental mice were killed at 5–8 wk of age.

Antibodies. Antibodies used for cell separation were FITC-labeled anti-CD19 (1D3), FITC-labeled anti–I-A^b (AF6–120.1), FITC-labeled anti-CD4 (RM4–5), FITC-labeled anti-CD8α (53–6.7), and FITC-labeled anti–TCR-β (H57–597), all of which were purchased from BD PharMingen. Antibodies used for stimulation and biochemical analysis were anti-γδTCR (UC7–13D5 and GL4), anti–TCR-β (H57–597), and CD3ε (145–2C11) (BD PharMingen), hamster anti–TCR-ζ (H146) and hamster anti-CD3γ (H25) (provided by D. Wiest, Fox Chase Cancer Center, Philadelphia, PA), rabbit anti-CD3δ (R9) (provided by L. Samelson, National Institutes of Health, Bethesda, MD), goat anti-CD3γ and goat anti-CD3ε (Santa Cruz Biotechnology, Inc.), HRP-conjugated Protein A (Transduction Laboratories), and donkey anti–goat IgG (H + L)-HRP (Jackson ImmunoResearch Laboratories).

Isolation of iIELs. iIELs were isolated as described previously (12).

Cell Separation. Peripheral αβ and γδ T cells were purified from lymph node cells from B6 and γδ TCR Tg mice, respectively, using the MACS® magnetic bead separation system (Miltenyi Biotec) as described previously (6). The purity of the resulting cell populations was typically 98% for αβ T cells and 99% for γδ T cells.

Stimulation and Expansion of T Cells In Vitro. 3×10^6 unfractionated lymph node cells were stimulated on 6-well plates coated with anti-γδTCR mAb (10 μg/ml GL4), anti–TCR-β mAb (10 μg/ml H57–597), or anti-Vγ1 mAb (10 μg/ml 2.11; reference 13) for 2 d and then expanded in the presence of 40 U/ml of murine rIL-2 (Peprotech, Inc.) for 2–12 more days to replicate conditions used in previous studies (2, 6). Unless otherwise noted, stimulated αβ and γδ T cells were harvested and analyzed 6 d after activation. Phenotypic analysis on day 6 revealed that cultures stimulated with anti-γδTCR mAb or with anti-Vγ1 mAb were 100% γδ TCR$^+$ or Vγ1$^+$, respectively. Similarly, cultures of B6 and CD3δ$^{-/-}$ lymph node cells stimulated with anti-αβTCR mAb were 100% αβ TCR$^+$ and were predominantly CD4$^-$ CD8$^+$. The only major difference detected between the B6 and CD3δ$^{-/-}$–stimulated αβ T cells was in the level of αβ TCR expression, with the level of surface TCR on CD3δ$^{-/-}$ αβ T cells being seven to eightfold less than that observed on B6 αβ T cells.

Surface Biotinylation. Biotinylation of surface proteins on purified αβ and γδ T cells was performed as described previously (6).

Deglycosylation of TCR Subunits. Removal of *N*-linked glycans from glycoproteins in the TCR complex has been described previously (14). In brief, immunoprecipitated TCR proteins were denatured by boiling in 1% SDS/1% 2-mercaptoethanol for 5 min. The eluted proteins were neutralized by the addition of 10% NP-40 and then resuspended in the reaction buffer provided by the manufacturer (New England Biolabs). Each sample was treated with 2,500 U peptide-*N*-glycosidase F (PNGase F; New England Biolabs) and then allowed to incubate overnight at room temperature. The digestion reaction was quenched with 2× SDS sample buffer containing 2-mercaptoethanol.

Protein Analysis. Immunoprecipitations and immunoblotting were performed as described previously (15), except that cells were lysed in a buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4, protease inhibitors (Roche Laboratories), and 1% Triton X-100 (Calbiochem). Two-dimensional (2-D) SDS-PAGE analysis of TCR subunits has been described previously (6). Biotinylated surface proteins were detected using ABC-HRP (Vector Laboratories) and the ECL Western blot detection system (Amersham Pharmacia Biotech).

Results and Discussion

Alteration in the CD3 Subunit Composition of the γδ TCR After TCR Stimulation. To determine whether in vitro activation and expansion lead to changes in the CD3 subunit composition of the γδ TCR, we compared the subunit composition of surface CD3 complexes expressed by primary and in vitro activated and expanded γδ T cells from V_γ6-J_γ1-C_γ1/V_δ1-D_δ2-J_δ2-C_δ γδ TCR Tg (γδ TCR Tg) mice (10). As shown in Fig. 1, 2-D SDS-PAGE analysis of anti-γδTCR immunoprecipitates revealed the presence of a 26-kD protein in CD3 complexes expressed on stimulated but not on unstimulated ex vivo γδ T cells. This protein was also observed in surface CD3 complexes when anti–TCR-ζ and anti-CD3ε mAbs (Fig. 1 and unpublished data) were used to immunoprecipitate TCR complexes from stimulated γδ T cells. Examination of the kinetics of appearance of the 26-kD protein in the γδ TCR after TCR activation and expansion showed that it first appeared in surface γδ TCRs at 5 d after activation and was still observed at the latest time point of the experiment (12 d after activation; unpublished data). The 26-kD protein was also detected in the CD3 complexes expressed by polyclonal γδ T cell populations from TCR-β$^{-/-}$ mice after TCR activation and expansion (unpublished data). Comparison of

*Abbreviations used in this paper: 2-D, two-dimensional; FcRγ, FcεR1 γ chain; iIEL, intestinal intraepithelial lymphocyte; PNGase, peptide-*N*-glycosidase.

the CD3 complexes on stimulated αβ and γδ T cells by 2-D SDS-PAGE analysis demonstrated that the 26-kD protein, detected in TCR complexes on activated γδ T cells, migrated similarly to CD3δ (Fig. 1). Therefore, these results demonstrated that activation and expansion induce the inclusion of a protein, comparable in mass and mobility to CD3δ, in the γδ TCR.

CD3δ Is Not a Component of the TCR Expressed by In Vitro Activated and Expanded γδ T Cells. To determine whether the 26-kD protein in the γδ TCR was in fact CD3δ, fully assembled TCR complexes from stimulated γδ T cells were recovered by immunoprecipitation with anti–TCR-ζ mAb and then immunoblotted with anti-CD3δ serum. As shown previously (6) and in Fig. 2 A, little or no CD3δ was detected in anti–TCR-ζ immunoprecipitates from ex vivo γδ T cells. In contrast, CD3δ was detected in anti–TCR-ζ immunoprecipitates from ex vivo αβ T cells (Fig. 2 A). Interestingly, the anti-CD3δ serum did not react with any protein in the γδ TCRs recovered from stimulated γδ T cells (Fig. 2 A). This finding is even more striking given the fact that total cellular CD3δ protein was increased in stimulated γδ T cell extracts relative to ex vivo γδ T cell extracts (Fig. 2 B). These data agree with those of our previous study, in which we reported that the TCR-γ and TCR-δ chains are unable to pair efficiently with CD3δε dimers (6).

As the TCR-γ chain in the transgenic γδ TCR utilizes the $C_\gamma1$ gene segment, we next determined whether TCR-γ chains using the $C_\gamma4$ gene segment (the other major C_γ gene segment expressed in peripheral γδ T cells; reference 13) could pair with CD3δε dimers. Using the anti-$V_\gamma1$ mAb (13), we selectively activated $V_\gamma1$-$J_\gamma4$-$C_\gamma4$–bearing γδ T cells from TCR-$\beta^{-/-}$ mice. 2-D SDS-PAGE analysis of surface biotinylated TCR subunits from stimu-

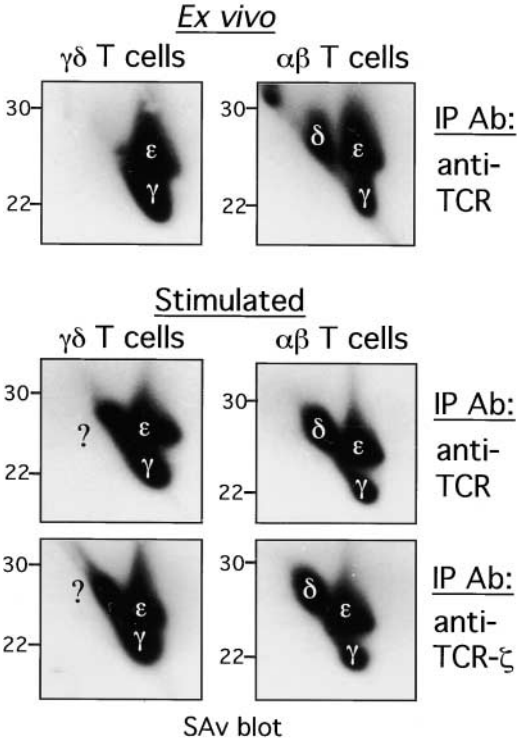

Figure 1. Effect of in vitro activation and expansion on the subunit composition of αβ- and γδ TCR complexes. Ex vivo αβ and γδ T cells were purified from the lymph nodes of B6 and γδ TCR Tg mice, respectively. Stimulated αβ and γδ T cells, from B6 and γδ TCR Tg mice, respectively, were generated as described in Materials and Methods. Surface proteins were labeled with biotin and αβ and γδ TCR complexes were immunoprecipitated using anti-TCR mAbs (H57–597 and UC7–13D5, respectively) or anti-TCR-ζ mAb (H146). Immunoprecipitated proteins were resolved by nonreducing/reducing 2-D SDS-PAGE. ABC-HRP and chemiluminescence were used to visualize surface biotinylated proteins. The positions of the CD3 subunits and the unknown 26-kD subunit (?) are marked.

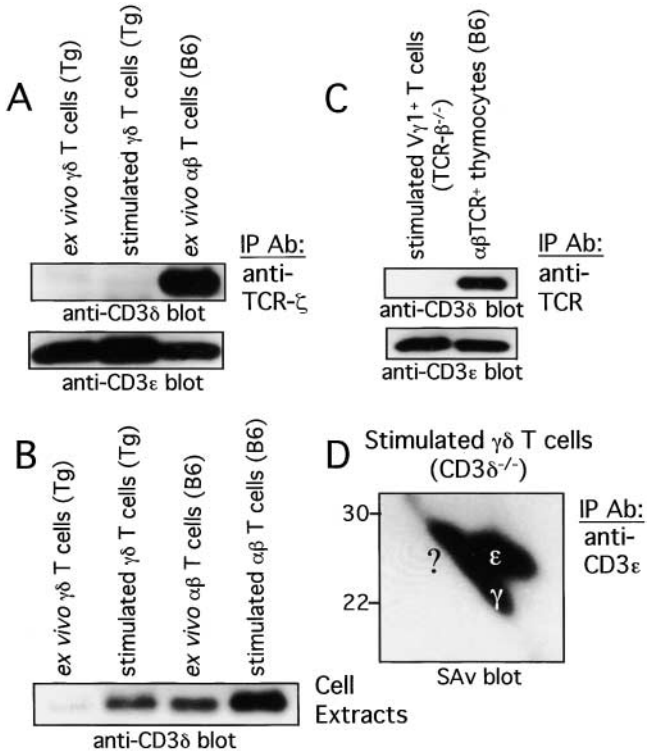

Figure 2. Biochemical analysis of CD3 complexes expressed by stimulated γδ T cells. (A) Lysates from 20×10^6 ex vivo αβ and γδ T cells and stimulated γδ T cells were immunoprecipitated with anti–TCR-ζ mAb (H146). Immunoprecipitated proteins were resolved by SDS-PAGE and immunoblotted with anti-CD3δ serum. The blot was subsequently stripped and probed with anti-CD3ε to assess the efficiency of immunoprecipitation. (B) Total cellular CD3δ protein levels in ex vivo and in stimulated αβ and γδ T cells. Extracts were made from 10×10^6 cells and 0.3×10^6 cell equivalents were then analyzed by immunoblotting with anti-CD3δ serum. (C) Lysates from 25×10^6 stimulated $V_\gamma1$-$J_\gamma4$-$C_\gamma4^+$ γδ T cells and B6 thymocytes were immunoprecipitated with anti-TCR mAbs (UC7–13D5 and H57–597, respectively). Immunoprecipitated proteins were resolved by SDS-PAGE and immunoblotted with anti-CD3δ serum. The blot was subsequently stripped and probed with anti-CD3ε to assess the efficiency of immunoprecipitation. (D) 20×10^6 stimulated γδ T cells from nontransgenic CD3$\delta^{-/-}$ mice were surface biotinylated, lysed, and incubated with anti-CD3ε mAb (145–2C11). Immunoprecipitated proteins were resolved by nonreducing/reducing 2-D SDS-PAGE. Biotinylated TCR subunits were detected with ABC-HRP and chemiluminescence. The positions of the CD3 subunits and the unknown 26-kD subunit (?) are marked.

lated $C_\gamma 4$-bearing γδ T cells also demonstrated the inclusion of a 26-kD protein in the γδ TCR after activation and expansion (unpublished data). However, this protein was not CD3δ as assessed by Western blot analysis of anti-γδ TCR immunoprecipitates (Fig. 2 C). Taken together, these results suggested that most γδ T cells, whether stimulated or unstimulated, do not contain CD3δ as a component of their TCRs.

To verify that the 26-kD protein detected in the γδ TCR after activation and expansion was not CD3δ, we analyzed the subunit composition of TCRs expressed by stimulated γδ T cells from $CD3\delta^{-/-}$ mice (11). Significantly, the surface CD3 complexes on stimulated $CD3\delta^{-/-}$ γδ T cells were similar in composition to those on stimulated $CD3\delta^{+/+}$ γδ T cells (Figs. 1 and 2 D, and unpublished data), thereby confirming that the 26-kD protein detected in the γδ TCR after TCR activation and expansion was not CD3δ. Based on these results, we conclude that activation and expansion induce changes in the CD3 complex of the γδ TCR but do not induce the inclusion of CD3δ.

CD3γ Is Modified After TCR Stimulation in Both αβ and γδ T Cells. The 26-kD protein identified in the γδ TCR after activation and expansion could potentially be a modified form of one of the other invariant TCR subunits, with the modification being differential *N*-glycosylation, ubiquitination, and/or phosphorylation. To determine whether this protein is a differentially glycosylated form of an invariant TCR subunit, we treated immunoprecipitated TCR proteins with PNGase F, a glycosidase that removes *N*-linked glycans at the asparagine residue. As shown in Fig. 3 A, three surface biotinylated proteins were detected in the CD3 complex in untreated anti-CD3ε immunoprecipitates from stimulated γδ T cells, as predicted by the 2-D SDS-PAGE analysis (Fig. 1). However, only two proteins, identical in mass to CD3ε and CD3γ, were detected in the CD3 complex after PNGase F treatment. This finding suggested that the 26-kD protein may be a differentially glycosylated form of CD3γ, because CD3γ is the only invariant subunit other than CD3δ to have an *N*-linked glycosylation site (16). To test this, we repeated the experiment using anti–TCR-ζ mAb to immunoprecipitate mature γδ TCR complexes and anti-CD3γ serum to immunoblot for CD3γ in the γδ TCR complex. In anti–TCR-ζ immunoprecipitates that were not treated with the glycosidase, we detected at least four forms of CD3γ, ranging in mass from 21–26-kD, in stimulated γδ T cells (Fig. 3 B).

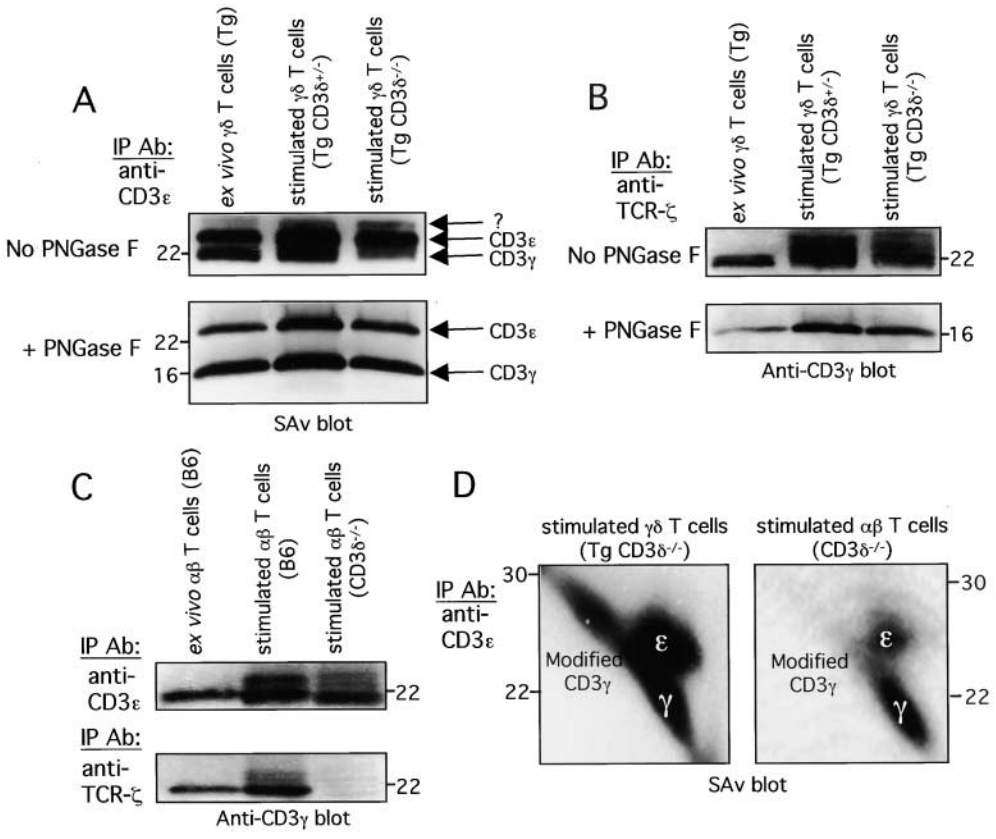

Figure 3. Modification of the CD3γ subunit after in vitro activation and expansion. (A) 30×10^6 ex vivo γδ (γδ TCR Tg [Tg] $CD3\delta^{+/-}$ mice) and stimulated γδ T cells (Tg $CD3\delta^{+/-}$ and Tg $CD3\delta^{-/-}$ mice) were surface biotinylated, lysed, and incubated with anti-CD3ε mAb (145–2C11). Immunoprecipitated proteins were either treated with PNGase F or left untreated and resolved by reducing SDS-PAGE. ABC-HRP and chemiluminescence were used to visualize surface biotinylated proteins. The positions of the CD3 subunits and the unknown 26-kD subunit (?) are marked. (B) Lysates from 30×10^6 ex vivo γδ (Tg $CD3\delta^{+/-}$ mice) and stimulated γδ T cells (Tg $CD3\delta^{+/-}$ and Tg $CD3\delta^{-/-}$ mice) were immunoprecipitated with anti-TCR-ζ mAb (H146) and then treated with PNGase F or left untreated. Digested and undigested TCR proteins were resolved by reducing SDS-PAGE and immunoblotted with anti-CD3γ serum. (C) Lysates from 30×10^6 ex vivo αβ (B6 mice) and stimulated αβ T cells (B6 and $CD3\delta^{-/-}$ mice) were immunoprecipitated with anti-CD3ε (145–2C11) or anti-TCR-ζ mAb (H146). Immunoprecipitated proteins were resolved by reducing SDS-PAGE and immunoblotted with anti-CD3γ serum. (D) Stimulated αβ and γδ T cells from $CD3\delta^{-/-}$ mice were generated as described in Materials and Methods. Surface proteins on equivalent numbers of cells were labeled with biotin and TCR complexes were immunoprecipitated using the anti-CD3ε mAb (145–2C11). Immunoprecipitated proteins were resolved by nonreducing/reducing 2-D SDS-PAGE. ABC-HRP and chemiluminescence were used to visualize surface biotinylated proteins. The exposure time for each blot is identical. The positions of the CD3ε and the modified forms of CD3γ are marked.

After PNGase F treatment, all forms of CD3γ were reduced to 16 kD, the core protein size of CD3γ. Interestingly, when we immunoprecipitated biotinylated TCR proteins with the anti-CD3γ mAb (H25), we did not detect the modified forms of CD3γ while we did detect the 21-kD form of CD3γ (unpublished data), suggesting that the differential glycosylation of CD3γ affects the binding of the H25 mAb. Therefore, these results suggested that the 26-kD protein in the CD3 complex of the γδ TCR is a differentially glycosylated form of CD3γ. As PNGase F treatment of this 26-kD protein removed only *N*-linked sugars and reduced its mass to 16 kD, these results indicate that CD3γ is modified only by differential glycosylation and not additionally by ubiquitination or phosphorylation, which are covalent modifications that are not affected by glycosidase treatment.

Because activation and expansion induced changes in the glycosylation pattern of the CD3γ subunit in γδ T cells, it was of particular interest to determine whether this modification also occurred in stimulated αβ T cells. To test this, we repeated the preceding experiment using αβ T cells that were activated and expanded in conditions similar to those used for γδ T cells. Western blot analysis was performed on anti-CD3ε immunoprecipitates from stimulated αβ T cells from CD3δ$^{+/+}$ mice. Interestingly, while differentially glycosylated forms of CD3γ were detected in stimulated αβ T cells, we did not observe the 26-kD form (Fig. 3 C). Next, we determined whether any of the modified forms of CD3γ were incorporated into fully assembled αβ TCRs by immunoblotting anti–TCR-ζ immunoprecipitates with anti-CD3γ serum. As shown in Fig. 3 C, only very low amounts of the modified forms of CD3γ were detected in fully assembled TCRs expressed by αβ T cells.

Because the TCRs on stimulated γδ T cells lack CD3δ and contain the differentially glycosylated forms of CD3γ, it is conceivable that the presence of CD3δ hinders the incorporation of modified CD3γ into the αβ TCR complex. To test this, we examined αβ TCRs from stimulated αβ T cells from CD3δ$^{-/-}$ mice for the presence of CD3γ modification. While we detected modified forms of CD3γ in CD3δ$^{-/-}$ αβ T cells, we did not detect any CD3γ (including the 21-kD form) associated with TCR-ζ (Fig. 3 C). The inability to detect CD3γ in the anti–TCR-ζ immunoprecipitate may be due to the instability and/or extremely low surface expression of CD3δ-deficient αβ TCR complexes (reference 11 and unpublished data). Therefore, we analyzed the subunit composition of αβ TCRs expressed on stimulated CD3δ$^{-/-}$ αβ T cells by surface biotinylation and 2-D SDS-PAGE. The amount of modified CD3γ detected in surface CD3δ-deficient αβ TCRs was negligible, similar to the amount detected in intact αβ TCRs (Figs. 1 and 3, C and D). These findings suggest that modified forms of CD3γ are major components of TCRs on stimulated γδ T cells but not on stimulated αβ T cells. The reason why these modified forms of CD3γ are incorporated at a high frequency into the fully assembled γδ TCR but not into the fully assembled αβ TCR is unknown, but it may be due to the ability of the TCR-γ/TCR-δ chains to bind more efficiently than the TCR-α/TCR-β chains to differentially glycosylated CD3γε dimers. This ability of the γδ TCR to accommodate CD3γε dimers with bulkier sugar groups demonstrates yet another fundamental difference in αβ and γδ TCR structure.

Modified CD3γ Is Detected in iIELs. While our results established that in vitro activation and expansion are able to induce modification of the CD3γ chain in both αβ and γδ T cells, it remained unclear whether this modification occurs in vivo. To address this, we analyzed the subunit composition of the TCRs expressed by iIELs, which are 50% γδ TCR$^+$ and display an activated phenotype (unpublished data and reference 17). As predicted from our analysis of TCR complexes on ex vivo and activated γδ T cells, little or no CD3δ was detected in TCR complexes on γδ TCR$^+$ iIELs (Fig. 4 A). When we assayed for modified forms of CD3γ, higher molecular weight forms of CD3γ were detected in anti-γδ TCR immunoprecipitates of iIEL lysates, albeit at a much lower level than that observed for in vitro activated and expanded γδ T cells (Figs. 4 A and 3 B).

The lower level of CD3γ modification may be due to the fact that γδ TCR$^+$ iIELs are not homogeneous in their state of activation and, consequently, there may be fewer cells that express the differentially glycosylated forms of CD3γ. To examine the extent of CD3γ modification in iIELs, we recovered CD3 dimers by immunoprecipitating unfractionated iIEL lysates with anti-CD3ε mAb and assayed for the presence of modified CD3γ to determine whether a change in the glycosylation pattern of CD3γ occurred in iIELs. As shown in Fig. 4 B, differentially glycosylated forms of CD3γ, but not the 26-kD form, were detected in iIELs (Fig. 4 B). These results demonstrated that

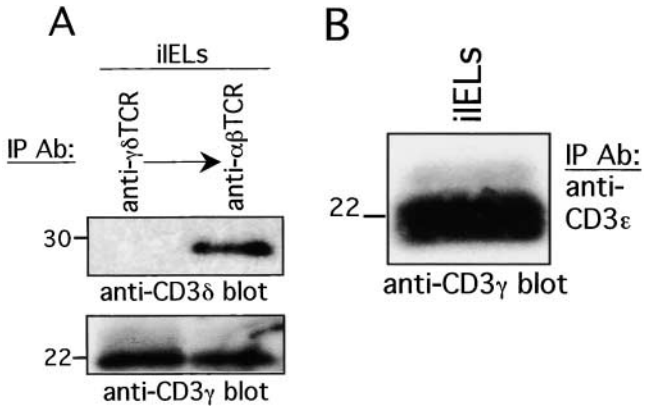

Figure 4. Detection of differentially glycosylated forms of CD3γ in iIELs. (A) Lysate from 30 × 10^6 iIELs from B6 mice was subjected to serial immunoprecipitations with anti-γδTCR and then anti-αβTCR mAbs (UC7–13D5 and H57–597, respectively). Immunoprecipitated proteins were resolved by reducing SDS-PAGE and immunoblotted with anti-CD3δ serum. The blot was subsequently stripped and immunoblotted with anti-CD3γ serum. (B) Lysates from 20 × 10^6 ex vivo iIELs from B6 mice were immunoprecipitated with anti-CD3ε (145–2C11). Immunoprecipitated proteins were resolved by reducing SDS-PAGE and immunoblotted with anti-CD3γ serum.

CD3γ modification is detected and therefore, can occur in activated mature T cells in vivo.

Many surface glycoproteins have been reported to undergo changes in glycosylation patterns during development and after activation (for reviews, see references 18 and 19). For example, two recent studies have shown that the *O*-linked sugars on CD8 play a role in cognate/noncognate binding of MHC class I tetramers to thymocytes (20, 21). The CD8 expressed on immature $CD4^+CD8^+$ thymocytes is capable of binding MHC class I tetramers from various MHC alleles and haplotypes. This is not the case for mature $CD8^+$ thymocytes and peripheral $CD8^+$ T cells, as their CD8 is sialylated and can only bind to the appropriate peptide–MHC class I complex (20, 21). Although the importance of this change in CD8 glycosylation is unknown, both groups speculated that the differential binding of CD8 molecules to MHC class I molecules during development may play a role in selection by affecting the span of time a developing thymocyte spends interacting with class I–bearing thymic stroma (20, 21).

Similarly, it is not known how the glycosylation status of CD3γ affects γδ TCR signaling and γδ T cell function. However, using mice with a targeted mutation in a carbohydrate-modifying enzyme as a guide (22), we can speculate as to the role of this modification in γδ T cell biology. It is known that a typical *N*-glycan is 30 Å in length, comparable to an Ig domain (23). Because in vitro activation and expansion increase the mass of the carbohydrate group on CD3γ, it is conceivable that the carbohydrate chain on CD3γ is elongated through addition of branched sugars (18). An increase in carbohydrate chain length on CD3γε dimers could cause the γδ TCR complexes in the cell membrane of stimulated γδ T cells to be spaced further apart, making it more difficult to cross-link surface γδ TCR complexes and thereby, increasing the threshold of signaling for the γδ TCR (22, 23). In practice, this may be a mechanism by which γδ T cells that recognize self-heat shock proteins or stress antigens establish a baseline threshold for signaling after their first encounter with self-antigens and then are able to distinguish between normal and stressed cells in subsequent encounters.

Modification in CD3γ glycosylation may not result in carbohydrate chain elongation but may result in the linkage of different sugar residues to the CD3γ chain. Such changes in carbohydrate moieties may affect the binding of endogenous lectins, such as the galectin family of carbohydrate-binding proteins, to the TCR complex (22). Because galectins have been shown to regulate T cell signaling and apoptosis (for a review, see reference 24), it is conceivable that the inclusion of modified CD3γ in the γδ TCR may facilitate the binding of galectins and increase the susceptibility of stimulated γδ T cells to apoptosis. If this is true, this may explain why, unlike αβ T cells, no memory response has been described for murine γδ T cells (for a review, see reference 25). While future studies are required to determine the importance of this modification, the fact that another alteration in γδ TCR subunit composition, inclusion of FcRγ, occurs after activation and expansion (6) suggests that the signaling potential of the γδ TCR may be profoundly affected.

In summary, we have described an activation and expansion induced modification in the CD3γ subunit of the TCR that consists of differential glycosylation at its single site of *N*-linked glycosylation. While this modification could occur in all T cells, the modified forms of CD3γ were only incorporated at a high frequency into the γδ TCR. One of the differentially glycosylated forms of CD3γ that was included in the γδ TCR had a mass and mobility in 2-D SDS-PAGE similar to CD3δ. We believe that it is this modified form of CD3γ that was initially identified as CD3δ in early studies of γδ TCR structure, because CD3δ is not detected in γδ TCR complexes on primary and in vitro activated and expanded γδ T cells.

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