Biochemical Evidence for the Presence of a Single CD3δ and CD3γ Chain in the Surface T Cell Receptor/CD3 Complex*

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The T cell antigen receptor (TCR) consists of an αβ heterodimer and associated invariant CD3γ, δ, ε, and ζ chains (TCR/CD3 complex). The general stoichiometry of the receptor complex, which is believed to be one molecule each of TCRα, TCRβ, CD3γ, and CD3δ and two molecules each of CD3ε and CD3ζ, is not clearly understood. Although it has been shown that there are two chains of CD3ε and CD3ζ, the stoichiometry of CD3γ or CD3δ chains in the surface antigen receptor complex has not been determined. In the present study, transgenic mice expressing an altered form of mouse CD3δ and CD3γ were employed to show that the surface TCR complexes contain one molecule each of CD3δ and CD3γ. Thymocytes from wild type and CD3 chain transgenic mice on the appropriate knockout background were surface-biotinylated and immunoprecipitated using a specific antibody. The immunoprecipitates were resolved in two dimensions under nonreducing/reducing conditions to determine the stoichiometry of CD3δ and CD3γ in the surface antigen receptor complex. Our data clearly show the presence of one molecule each of CD3δ and CD3γ in the surface TCR/CD3 complex.

The TCR/CD3 complex is multimeric and consists of a covalently linked heterodimer of polymorphic TCRα and β glycoproteins and noncovalently associated invariant CD3γ, δ, ε, and ζ chains. Individual CD3 chains contain immunoreceptor tyrosine-based activation motifs in their cytoplasmic domain. The TCR/CD3 receptor complex recognizes a peptide antigen presented by MHC molecules expressed on the surface of antigen-presenting cells. The engagement of the surface receptor complex to its ligand results in signal transduction and is most likely mediated by CD3 chains via phosphorylation of immunoreceptor tyrosine-based activation motifs (1, 2). The presence of multiple CD3 chains has been suggested to amplify a signal generated by TCR-peptide/MHC interactions resulting in diverse outcomes, for instance, positive versus negative selection of developing thymocytes. In addition to signal transduction, the CD3 components play a crucial role in the receptor complex assembly, stability, and transport to the cell surface as well as ligand-induced surface receptor internalization. Furthermore, it has been suggested that engagement of the surface receptor complex by a single molecule of peptide/MHC is sufficient to elicit a cytotoxic T cell response (3). A single peptide/MHC complex could engage multiple TCR complexes in a serial manner, as suggested by Lanzavecchia and colleagues (4, 5). This would imply that CD3 chains present in a single TCR complex are capable of recruiting sufficient numbers of intracellular signaling molecules, leading to T cell activation. This could be achieved by altering the conformation of the receptor, as has recently been shown by Alarcon and colleagues (6). Following TCR/ligand engagement, recruitment of an adaptor protein, Nck, to the proline-rich sequence in the cytoplasmic domain of CD3ε has been suggested to alter the conformation of the receptor complex, leading to T cell activation (6, 7).

Since CD3 chains are central to the TCR assembly, transport, and signal transduction, it is important to understand their stoichiometry in the receptor complex. The exact stoichiometry of all the components of the TCR/CD3 complex is not clear. A widely accepted octameric model of TCR/CD3 complex proposes that there are two molecules each of CD3ε and ζ chains and one molecule each of CD3γ, δ, TCRα, and β chains (8–12). The model is further supported by data from Al Singer and his group (13), who have suggested that CD3γε and CD3δϵ heterodimers preferentially associate with TCRβ and TCRα chains, respectively. Recent reports by Call et al. (14, 15) also support an octameric model of the TCR/CD3 complex. Using an in vitro translation system, these authors propose that a basic residue in the transmembrane domains of TCRα and TCRβ chain interact with acidic residues in the transmembrane domains of CD3ε and γε heterodimers, respectively (14, 15). Analysis of the assembled TCR/CD3 complex using this experimental system strongly supports the octameric model of TCR/CD3 stoichiometry. However, these data do not address the stoichiometry of the surface TCR/CD3 complex, which is central to the T cell development and function following ligand engagement. Further, cell-free systems do not address the possibility that processing of complex carbohydrates on glycoproteins (TCRα, TCRβ, CD3γ, and CD3δ are glycoproteins) may influence the stoichiometry of the surface complex. For instance, tunicamycin treatment of Jurkat T cells, which disrupts protein glycosylation, has been shown to prevent assembly of CD3δ into the receptor complex (16).

A possibility that the surface receptor complex may contain multivalent TCR/CD3 complexes has been supported by recent biochemical and crystallographic data. It has been shown that there could exist at least three molecules of CD3ε chain (17) and two molecules of TCRβ chain (and by extension, the TCRα chain) (17, 18). Based on these data, a decameric model of TCR/CD3 complex stoichiometry has been proposed (19). According to this model, there are two molecules each of CD3ε, CD3ζ, TCRα, and TCRβ chains and one molecule each of CD3γ and CD3δ. The presence of two molecules of each TCR chain

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The abbreviations used are: TCR, T cell antigen receptor; GS, γ stop; DS, δ stop; HRP, horseradish peroxidase; MHC, major histocompatibility complex.
was further supported by the crystal structure of variable domains of TCR chains. The crystal structure was shown to contain four TCR chains representing two heterodimers of TCRα and β chains (20, 21). Two TCR heterodimers were proposed to interact with a dimer of MHC class II complex (22). The presence of four TCR chains and their preferential association with specific CD3 heterodimers raises questions about the stoichiometry of CD3γ and CD3δ chains. These data suggest that there could be two chains each of CD3γ and CD3δ in the receptor complex. Both CD3γ and CD3δ are glycoproteins and play an important role in TCR assembly and signal transduction. Mice deficient in either CD3γ or CD3δ exhibit a blockade in development at the double negative and double positive stage, respectively (23–25). In the absence of CD3γ, the TCRαβCD3γε complex is formed quite efficiently (23). However, the CD3δ-deficient TCR complex failed to associate with CD3ε chain, resulting in an inefficient transfer to the cell surface (23). These data indicated that, at least intracellularly, CD3γ, and possibly CD3δ, could associate with either of the TCR chains. Thus, a small number of TCR/CD3 complex expressed on T cell surface in either CD3γ−/− or CD3δ−/− mice could contain more than one molecule of CD3δ or CD3γ, respectively. Similarly, cell line studies have shown that CD3γε and CD3δε heterodimers could associate with either of the TCR chains, further supporting the multivalent structure of the TCR/CD3 complex (26, 27). Irrespective of whether CD3γ and CD3δ chains associate preferentially or randomly with TCR chains, if there are two molecules each of TCRα and β chains, there exists a possibility that there could be two molecules each of CD3γ and CD3δ in a given TCR/CD3 complex. In this report, we have attempted to dissect the stoichiometry of CD3δ and CD3γ chains in the surface TCR/CD3 complex. Using CD3δ and CD3γ transgenic mice combined with specific immunoprecipitation of the surface-labeled TCR/CD3 complex, we show biochemically that the majority of the surface TCR/CD3 complexes contain a single chain of CD3δ and CD3γ per TCR complex.

MATERIALS AND METHODS

Mice—CD3δ−/− and CD3γ−/− mice have been described previously (23, 25). Truncated CD3δ (δ stop, or DS) and CD3γ (γ stop, or GS) transgenic constructs were engineered by amplifying mouse cDNA using specific primers. The primers were designed to introduce a stop codon immediately after the transmembrane region of the two proteins. The amplified cDNA products were cloned into a TA cloning vector, sequence-verified subsequent to subcloning into a human CD3ε expression cassette, which ensures expression throughout T cell ontogeny (28). The final construct was separated from the vector sequence by restriction enzyme digestion, gel-purified, and injected into fertilized mouse eggs. Founder mice were identified by Southern blot and/or PCR techniques. Initially, DS and GS transgenics were introduced in CD3δ−/− and CD3γ−/− background, respectively. The DS δ−/− and GS γ−/− transgenic mice were backcrossed for 2–3 generations to obtain DS δ−/− (DS δ−/−) or DS GS γ−/− mice. C57B1/6 mice were used as control. All mice were bred and maintained in a specific pathogen-free animal facility at the IRCM.

Antibodies—2C11 is a hamster monoclonal antibody that recognizes mouse CD3ε and CD3ε heterodimers and was purified from hybridoma culture supernatant on a protein A-Sepharose column (Amerham Biosciences). Rabbit polyclonal serum, R9, was a kind gift of Lawrence Samelson (National Institutes of Health, Bethesda, MD). R9 recognizes the cytoplasmic tail of 26-kDa mouse and human CD3δ protein. Anti-CD3γ and anti-CD3ε rabbit polyclonal sera were a generous gift of Ed Palmer (University Hospital-Basel, Switzerland). HMT3.2 antibody, which recognizes the cytoplasmic domain of CD3γ and CD3δ, was a gift from Balbino Alarcon (Universidad Autonoma de Madrid, Madrid, Spain) or purified from hybridoma culture supernatant. Anti-CD4-phycocerythrine, anti-CD8-fluorescein isothiocyanate, anti-TCR-cy whole antibodies were obtained from Pharmingen. Purified rabbit anti-mouse IgG, goat anti-mouse IgG fluorescence isothiocyanate, and goat anti-mouse IgG-cyanine5 antibodies were obtained from Jackson ImmunoResearch, West Grove, PA. Thymocytes and lymph node cells were stained with a panel of antibodies and analyzed using a FACS Caliber.

Immunoprecipitation and Western Blot—One-two hundred million thymocytes were lysed in 1% digitonin or Triton X-100 lysis buffer containing 50 mM Tris, pH 7.6, 150 mM NaCl, 50 mM NaF, 1 μM EDTA, leupeptin, 1 μg/ml pepstatin, leupeptin, phenylmethylsulfonyl fluoride, N-α-tosyl-L-lysine chloromethyl ketone hydrochloride). Cells were lysed for 45 min on ice followed by centrifugation at 13,000 rpm for 5 min at 4 °C. The post-nuclear supernatant was incubated with protein A-Sepharose beads for 1 h at 4 °C on a rotating wheel. The precleared lysate was immunoprecipitated using antibody-coated protein A-Sepharose beads. The beads were washed twice with ice-cold wash buffer (50 mM Tris, pH 7.6, 150 mM NaCl, 0.2% detergent) followed by ice-cold phosphate-buffered saline. Immunoprecipitates were eluted in the protein sample loading buffer (62.5 mM Tris-HCl, pH 6.7, 2% SDS, and 100 mM 2-mercaptoethanol at 50 °C for 1 h, washed 4–6 times in Tris-HCl buffer, and repointed.

Surface Biotinylation—Thymocytes were washed in Hank’s balanced salt solution (Invitrogen) containing 1 mM MgCl2 and 0.1 mM CaCl2, suspended in 1 ml of wash buffer and cooled on ice for 20 min. Surface proteins were biotinylated for 20 min on ice by adding sulfo-N-hydroxysuccinimidobiotin (Pierce) to a final concentration of 500 μg/ml. The biotinylation reaction was terminated by adding Hank’s balanced salt solution containing 25 mM lysine monochloride. The biotylated thymocytes were washed twice with lysine monochloride/Hank’s buffered saline solution, and the viability of the labeled cells was determined by trypan blue exclusion. More than 98% of the labeled thymocytes were found to be viable. Cells were lysed, immunoprecipitated as described above, and separated on a 13% tube gel under nonreducing conditions. The tube gels were equilibrated with protein loading dye containing 100 mM 2-mercaptoethanol at 57 °C with mild shaking. The tube gels were then loaded onto 13% SDS-PAGE gel for electrophoresis in the second dimension. The resolved proteins were transferred onto Immobilon membrane, blocked in 5% milk/phosphate-buffered saline, washed, and probed with HRP-avidin (Sigma). Bands were visualized with the chemiluminescence system described above.

RESULTS

Mouse Surface TCR Complexes Contain Only One Molecule of CD3δ Protein—To test the stoichiometry of mouse CD3δ, we took advantage of CD3γ-deficient, cytoplasmic domain truncated CD3δ (DS) and CD3δ transgenic (GS) mice. As mentioned earlier, CD3γ and CD3δ deficiency blocks thymocyte development at the double negative and double positive stage, respectively. This suggests that these two mouse proteins, although homologous to each other, do not substitute for each other for thymocyte maturation.

We have shown that the DS transgene restores thymocyte development in CD3δ-deficient mice (24). Likewise, the GS transgene restores thymocyte development in CD3γ-deficient mice.2 To test the stoichiometry of CD3δ, we introduced the DS and GS transgenes into a CD3γ-deficient background. Thus, the DS GS γ−/− mouse expresses two forms of the CD3δ chain, one full-length endogenous and one truncated transgenic. The presence of a truncated form of the CD3γ chain in the CD3γ-deficient background ensures that thymocyte development is normal and that these cells express only one form of the CD3γ chain. Since one of the CD3δ molecules is devoid of the cytoplasmic tail, it is not possible to test the presence of two CD3δ chains in immunoprecipitates of total lysates in Western blot using R9 serum (as the serum cannot recognize the DS protein). Further, since the engagement of the surface receptor complex to its ligand is crucial for T cell development and function, it is important to dissect the stoichiometry of the

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CD3δ and CD3γ Stoichiometry

Fig. 1. Monomeric stoichiometry of CD3δ. A, schematic presentation of the possible stoichiometry of CD3δ in DS'GS'γ−/− mice and the number of bands expected on avidin blots of HMT3.2 and 2C11 immunoprecipitates of surface-biotinylated receptor complexes. For the sake of simplicity, only CD3 γ, δ, and ε chain stoichiometry is shown. HMT3.2 recognizes the cytoplasmic tail of CD3δ, and thus, will immunoprecipitate only those receptor complexes containing endogenous CD3δ. Thus, if CD3δ exists as a monomer, it will generate endogenous CD3δ containing...
The presence of only the endogenous, but not truncated, CD3\(\gamma\) shows a schematic of possible outcomes of HMT3.2 and 2C11 genes into CD3 complexes indeed contain only one molecule each of CD3\(\gamma\) and CD3\(\delta\) (30). Accordingly, the antibody will immunoprecipitate only those TCR complexes that contain endogenous CD3\(\delta\). Fig. 1A shows a schematic of possible outcomes of HMT3.2 and 2C11 immunoprecipitation of only DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) thymocytes. Thus, HMT3.2 immunoprecipitates will show either three or four DS-specific bands depending on whether there are one or two chains of CD3\(\delta\) in a TCR complex. The immunoprecipitates were resolved under nonreduced and reduced conditions in the first and second dimensions, respectively. As shown in Fig. 1B, panel C, HMT3.2 immunoprecipitates of the DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) thymocytes show GS-, CD3\(\epsilon\)-, and CD3\(\delta\)-specific, but not DS-specific, bands (for simplicity, only CD3-specific bands are shown in all the figures). In contrast, 2C11 immunoprecipitates of the DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) thymocytes show GS-, CD3\(\epsilon\)-, and CD3\(\delta\)-specific bands (Fig. 1C, panel C). Comparable amounts of endogenous CD3\(\delta\) and DS proteins could be immunoprecipitated with 2C11. Immunoprecipitates of wild type and GS\(^{+}\)\(\gamma\)\(\delta\) mice show the position of endogenous proteins and the truncated protein with both the antibodies (Fig. 1, B and C, panel B). The presence of only the endogenous, but not truncated, CD3\(\delta\) in HMT3.2 immunoprecipitates strongly indicates that there is only one CD3\(\delta\) chain per TCR complex.

**CD3\(\gamma\) Is Also Present as a Monomer in the Surface TCR/CD3 Complexes**—The above described data clearly show that the majority of TCR complexes contain only a single molecule of the CD3\(\delta\) chain. To test whether a TCR complex contains one molecule of CD3\(\gamma\) as well, we introduced the DS and GS transgenes into DS\(^{+}\)GS\(^{+}\) mice. Thus, T cells from DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) mice will express two forms of CD3\(\delta\) (one endogenous and one truncated) and only a truncated form of CD3\(\gamma\). Fig. 2A shows a schematic of the CD3\(\delta\) stoichiometry experiment. In this case, HMT3.2 will immunoprecipitate only those TCR complexes that contain the endogenous CD3\(\delta\) (Fig. 2A). Thus, we immunoprecipitated surface-biotinylated thymocytes from wild type, DS\(^{+}\)\(\gamma\)\(\delta\), and DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) mice with HMT3.2 or 2C11. As shown in Fig. 2B, panel C, HMT3.2 immunoprecipitates of the DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) thymocytes show only full-length CD3\(\gamma\) along with truncated CD3\(\delta\) and endogenous CD3\(\delta\) proteins. As expected, 2C11 immunoprecipitates all four proteins, which are wild type CD3\(\gamma\), GS, DS, and CD3\(\epsilon\), from the lysate of DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) thymocytes (Fig. 2C, panel C). As shown here, comparable amounts of endogenous CD3\(\gamma\) and GS proteins could be immunoprecipitated with 2C11. The presence of only endogenous CD3\(\delta\) in HMT3.2 immunoprecipitates suggests the existence of one CD3\(\gamma\) chain per TCR complex. All these data indicate that the surface TCR complex contains only one molecule each of CD3\(\delta\) and CD3\(\gamma\).

Although the above data strongly suggest that surface TCR complexes contain only one chain each of CD3\(\delta\) and CD3\(\gamma\), we wished to ascertain that this was not due to the experimental conditions employed. We predicted that if the surface TCR complexes indeed contain only one molecule each of CD3\(\delta\) and CD3\(\gamma\), then it should be possible to immunoprecipitate two sets of TCR/CD3 complexes from the double transgenic mice. One set of TCR complexes will contain endogenous CD3\(\delta\), and the other set will contain the truncated CD3\(\delta\) (DS) on DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) thymocytes (Fig. 1A). Similarly, TCR complexes will contain either endogenous or truncated CD3\(\gamma\) on DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) thymocytes (Fig. 2A). Thus, we should be able to immunoprecipitate all of the endogenous CD3\(\delta\)-containing complexes with HMT3.2 and the remaining complexes containing truncated CD3\(\delta\) with 2C11. To test this, we carried out sequential immunoprecipitation of biotinylated surface TCR/CD3 complexes. Lysates of biotinylated surface receptor complexes were immunoprecipitated sequentially four times with HMT3.2 antibody and finally with 2C11. Fig. 3 shows avidin blots of the first HMT3.2 and final 2C11 sequential immunoprecipitations of wild type (panels A and B), DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) (panels C and D), and DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) (panels E and F) thymocytes. As shown, HMT3.2 immunoprecipitated only wild type CD3\(\delta\)-, GS-, and CD3\(\epsilon\)-containing TCR complexes from DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) thymocytes (panel C). Similarly, HMT3.2 immunoprecipitated only wild type CD3\(\gamma\)-, DS-, and CD3\(\epsilon\)-containing TCR complexes from DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) (panel E). The remaining TCR complexes containing only the truncated proteins along with CD3\(\epsilon\) from DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) and DS\(^{+}\)GS\(^{+}\)\(\gamma\)\(\delta\) thymocytes could be immunoprecipitated by 2C11 (panels D and F).

**DISCUSSION**

In the present investigation, we have attempted to address the stoichiometry of CD3\(\delta\) and CD3\(\gamma\) in the surface TCR/CD3 complex. CD3\(\delta\) and CD3\(\gamma\) play an important role in intracellular assembly and transport of TCR/CD3 complexes to the cell surface as well as signal transduction and receptor internalization following ligand engagement. Several reports suggest that TCR\(\alpha\) and TCR\(\beta\) chains preferentially associate with CD3\(\delta\) and CD3\(\epsilon\) heterodimers, respectively (8–13), whereas others have shown that TCR chains can associate indiscriminately with either CD3\(\delta\) or CD3\(\epsilon\) subcomplexes (18, 26, 27). Based on the preferential TCR/CD3 chain association, an octameric model of TCR/CD3 stoichiometry has been suggested, which predicts two molecules each of CD3\(\epsilon\) and \(\gamma\) and one each of TCR\(\alpha\), \(\beta\), CD3\(\gamma\), and \(\delta\) per TCR complex. However, the crystal structure of TCR chains suggests that TCR\(\alpha\) and -\(\beta\) chains could exist as two heterodimers supporting a multivalent structure for the TCR/CD3 complex (20, 21). This was further supported biochemically and by surface modulation of TCR chains. Using double TCR transgenic mice, it was shown that two molecules of transgenic TCR\(\beta\) chains encoding different specificities could exist in a given TCR/CD3 complex (18). Cell line studies have also indicated that TCR\(\alpha\) and -\(\beta\) chains can indiscriminately associate with either CD3\(\delta\) or CD3\(\epsilon\) (26, 27). All these data point to a multivalent structure for TCR/CD3 complex (19). These data in turn imply that there could exist two molecules of CD3\(\delta\) and CD3\(\gamma\) in a TCR complex.

To elucidate the stoichiometry of CD3\(\delta\) and CD3\(\gamma\), we made use of truncated CD3\(\delta\) (GS) and CD3\(\delta\) (DS) transgenes. While the DS transgene rescues thymic selection in CD3\(\delta\)-deficient mice (24), the GS transgene restores early thymocyte matura-
FIG. 2. Monomeric stoichiometry of CD3γ. A, schematic presentation of the possible stoichiometry of CD3γ in DS'GS'δ−/− mice and the number of bands expected on avidin blots of HMT3.2 and 2C11 immunoprecipitates of surface-biotinylated receptor complexes. The presence of only one chain of CD3γ per TCR complex will result in two types of TCR/CD3 complexes in DS'GS'δ−/− mice. HMT3.2 will immunoprecipitate only type-A TCR complexes containing endogenous CD3γ resulting in three bands (CD3γ, DS, and CD3e) on an avidin blot. On the other hand, dimeric CD3γ stoichiometry will result in three types of receptor complexes (type-C, -D, and -E). HMT3.2 will immunoprecipitate endogenous CD3γ containing type-C and -D complexes resulting in four bands (CD3γ, DS, GS, and CD3e) on an avidin blot. 2C11 immunoprecipitate will show four CD3 chain-specific bands in both situations. B and C, the presence of a single molecule of CD3γ in the surface TCR complex. Thymocytes from wild type (WT), DS'δ−/−, and DS'GS'δ−/− mice were surface-biotinylated, lysed, and immunoprecipitated using either HMT3.2 (B) or 2C11 (C), resolved on a two-dimensional gel, transferred onto nylon membrane, and probed with HRP-avidin and ECL. HMT3.2 immunoprecipitates show three bands, indicating the presence of one molecule of CD3γ per TCR complex. As expected, 2C11 immunoprecipitates show four bands.
Thus, we generated DS test the stoichiometry of CD3ε and CD3δ. Our specific immunoprecipitation data clearly indicate that the surface TCR complexes contain a single molecule each of CD3γ and CD3δ. That TCR complexes contain only one molecule each of CD3γ and CD3δ was confirmed by sequential immunoprecipitates data shown in Fig. 3, which clearly show that two types of TCR complexes can be immunoprecipitated from double transgenic thymocytes. Thus, our data unequivocally show the presence of only one molecule each of CD3γ and CD3δ in the surface TCR complex. We would like to emphasize that data described in this report are relevant to receptor complexes containing αβ TCR heterodimers. Receptor complexes containing γδ TCR heterodimers have been shown to lack CD3ε chain and are associated with differentially glycosylated forms of CD3γ when activated under ex vivo conditions (29, 30).

Although data reported by Call et al. (14, 15) support the monomeric CD3δ and CD3γ stoichiometry, it is important to point out that this was achieved by immunoprecipitation of receptor complexes synthesized using a cell-free in vitro translation system. However, this system cannot be employed to test the stoichiometry of surface receptor complexes, which could be influenced by accessory molecules, for instance, CD4 and CD8 coreceptor molecules (31, 32). Also, glycosylation of CD3δ, but not CD3γ, has been shown to be necessary for its assembly into the TCR complex of the Jurkat T cell line (16). Our data obtained using surface biotinylation of TCR/CD3 complexes combined with specific immunoprecipitation clearly show the presence of only one CD3γ and CD3δ chain in surface TCR/CD3 complexes. In all the HMT3.2 immunoprecipitation experiments carried out, we consistently observed only endogenous, but not truncated, CD3δ in DS’GS’γ’’ and endogenous CD3δ. That TCR complexes contain only one molecule each of CD3γ and CD3δ proteins in the TCR complexes. This was confirmed in sequential immunoprecipitation experiments wherein the final 2C11 immunoprecipitates also showed three CD3-specific bands (versus four bands in direct 2C11 immunoprecipitates). In conclusion, our data clearly show the presence of one chain each of CD3γ and CD3δ in the surface αβ TCR complex.

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Fig. 3. Sequential immunoprecipitation of TCR/CD3 complexes from DS’GS’γ’’ and DS’GS’δ’’ mice supports a stoichiometry of one CD3δ and one CD3γ chain per TCR complex. Surface-biotinylated TCR complexes from wild type (WT), DS’ GS’γ’’, and DS’ GS’δ’’ mice were immunoprecipitated four times with HMT3.2 antibody followed by 2C11 antibody. The first HMT3.2 (A, C, and E) and final 2C11 (B, D, and F) immunoprecipitates of wild type (A and B), DS’ GS’γ’’ (C and D), and DS’ GS’δ’’ (E and F) thymocytes were resolved on a two-dimensional gel, blotted, and probed with HRP-avidin. TCR/CD3 complexes containing only the endogenous CD3δ or CD3γ could be immunoprecipitated by HMT3.2 antibody (C and E), whereas those containing only the truncated proteins could be immunoprecipitated by 2C11 (D and F) from lysates of double transgenic thymocytes as shown by the detection of only three bands in all the immunoprecipitates. The absence of any bands in 2C11 immunoprecipitates of wild type mice (B) indicates the complete preclearing of endogenous CD3δ- and/or CD3γ-containing TCR complexes by HMT3.2. These data clearly show that the surface receptors contain one chain of CD3γ and CD3δ per TCR complex.
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