Brief Definitive Report

Preferential Usage of the Fc Receptor γ Chain in the T Cell Antigen Receptor Complex by γ/δ T Cells Localized in Epithelia

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Summary

ζ and η chains of the T cell antigen receptor (TCR) complex and the γ chain of Fc receptors (FcRγ) constitute a family of proteins important for the expression of, and signal transduction through, these receptors in hematopoietic cells. In ζ-deficient mice, TCR expression was reduced in most T cells. By contrast, CD8$\alpha\alpha^+$ TCR-γ/δ^+ intestinal intraepithelial lymphocytes in these mice expressed a normal level of TCR. Biochemical analysis of the TCR complex in these cells from ζ-deficient as well as normal mice revealed the predominant usage of FcRγ. Furthermore, γ/δ^+ T cells in epithelia of the skin and female reproductive organs from ζ-deficient mice also showed relatively high TCR expression, indicating the usage of FcRγ. These observations demonstrate the preferential usage of FcRγ by γ/δ^+ T cells localized in epithelia of normal mice.

T cells recognize antigen bound to the products of MHC on APC through TCR, and are activated to exert various effector functions (1, 2). The TCR complex is a multimeric complex composed of three groups of proteins; the TCR-α/β (or -γ/δ) dimer, the CD3 complex, and the ζ family of disulfide-linked dimers (3). TCR dimers are responsible for antigen binding, whereas the other molecules are thought to be important for transmembrane signaling.

The ζ family has three known members, ζ, η, and FcRγ (4–6). In thymocytes and peripheral T cells, most of TCR complexes contain ζ homodimers, whereas 5–10% of TCRs associate with the ζ–η heterodimer. On the other hand, the TCR complex in a minor population of T cells has ζ and η as a form of heterodimers with FcRγ (4, 5). Moreover, it is reported that FcRγ was exclusively associated with TCR in in vitro cultured large granular lymphocytes (6).

A considerable body of evidence shows the importance of ζ both in assembly and surface expression of the TCR complex and in receptor-mediated signal transduction (7–13). Cytoplasmic tails of ζ family as well as CD3 molecules have consensus motifs important for intracellular signal transduction (14, 15). Recent studies indicate that the TCR complex has two signal transduction modules, the ζ family dimer and the CD3 complex, and that the two modules could transduce distinct signals into the cells (15, 16). In addition, it has been reported that the TCR complex containing FcRγ has a different signaling capacity from those containing ζ (17–19). Consequently, differential usage of ζ family molecules by distinct subsets of T cells may reflect difference in lineage and/or function of these cells.

Recently, we and others demonstrated that ζ is critical for normal T cell development and function, using mice deficient in the expression of ζ (20, 21). We also showed that η is not so efficient in assembly and surface expression of TCR complexes as ζ (20). Biochemical analysis of T hybridoma cells revealed that this was caused by retention in the endoplasmic reticulum of η-containing TCR complexes (22). Taking advantage of the mice lacking ζ (ζT/ζT mice) (20), we evaluated FcRγ usage by various subsets of T cells.

Materials and Methods

Mice. C57BL/6 and KSN *nu/nu* mice (23) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). ζT mice (20) were bred in our facility.

Cell Preparation. Single cell suspensions of thymocytes and splenocytes were prepared in RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM glutamine, 50 μM 2-ME, and 100 μg/ml kanamycin. Splenocytes were depleted of erythrocytes by lysis. For enrichment of CD4$^-$8$^-$ double-negative (DN) thymocytes, thymocytes were incubated with culture supernatant of anti-CD4 mAb (MT4), plated on plastic dishes precoated with rabbit anti-mouse immunoglobulin (Cappel Laboratories, Cochranville, PA), and nonadherent cells were collected as DN thymocytes-enriched population. Intestinal intraepithelial lymphocytes (i-IEL), hepatic lymphocytes, lymphocytes in mucosal epithelia of

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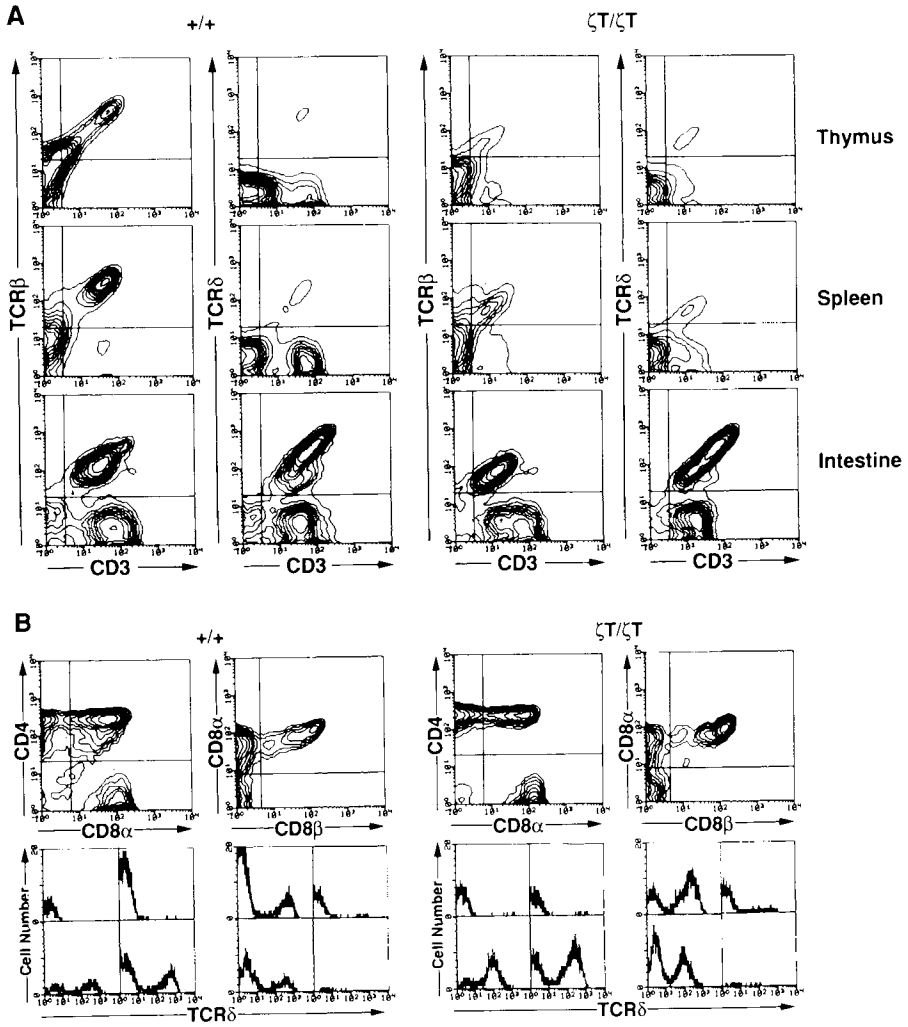

Figure 1. Expression of TCR-α/β and -γ/δ dimers on thymocytes, splenocytes, and i-IEL from $\zeta T/\zeta T$ mice. (*A*) CD3ϵ and TCRβ or TCRδ expression on the surface of thymocytes, splenocytes, and i-IEL were examined as described in Materials and Methods. (*B*) CD4, CD8, and TCRδ expression on the surface of i-IEL were analyzed. CD4/CD8α or CD8α/CD8β were used to divide i-IEL into four subpopulations (*top*). The histograms shown below represent the fluorescence intensity for TCR-δ detected by the third fluorescence, within each quadrant.

the female reproductive tract (r-IEL), and Thy-1$^+$ dendritic epidermal T cells (DETC) were isolated as previously described (24–27).

Flow Cytometric Analysis. Cells from the thymus, spleen, peripheral blood, liver, i-IEL, DETC, and r-IEL were stained with mAbs recognizing CD3ϵ, TCR-α/β, TCR-γ/δ, CD4, CD8α, CD8β, and IL-2Rβ, and the surface expression of these molecules was analyzed on a FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA). The following mAbs were used: PE-conjugated anti-CD4 (GK1.5) and FITC-labeled anti-CD8α (53-6.7), purchased from Becton Dickinson & Co., FITC-conjugated or biotinylated anti-CD3ϵ (145-2C11); biotinylated anti-TCR-β (H57-597), biotinylated anti-TCR-γ/δ (GL3); and PE-labeled anti-CD45R/B220 (RA3-6B2) from PharMingen (San Diego, CA); biotinylated anti-IL-2Rβ (TM-β1) was kindly provided by Dr. M. Miyasaka (Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan). Biotinylated mAbs were developed with streptavidin-PE (Becton Dickinson & Co.) or streptavidin-Tri-Color® (Caltag Laboratories, San Francisco, CA). Dead cells were excluded by staining with propidium iodide. Cells in the lymphocyte gate defined by light scatter were collected.

Cell Surface Biotinylation, Immunoprecipitation, and Two-dimensional SDS-PAGE Analysis. Cell surface biotinylation was performed as previously described (28). Cells were then solubilized in lysis buffer (1% digitonin, 50 mM Tris-HCl, pH 7.6, 300 mM NaCl, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM PMSF, 10 mM iodoacetamide) at a concentration of 10^8 cells/ml. Immunoprecipitation was performed with anti-TCR-δ mAb (3A10), anti-TCR-β mAb (H57-597), anti-ζ mAb (H146.698-A), and anti-FcRγ antiserum, which were kindly provided by Drs. S. Tonegawa (Massachusetts Institute of Technology, Boston, MA), R. Kubo (Cytel Corporation, San Diego, CA), and C. Ra (Juntendo University, Tokyo, Japan), respectively. Immunoprecipitates were resolved by two-dimensional nonreducing-reducing SDS-PAGE (14% for the first dimension and 16% for the second dimension), transferred onto polyvinylidene fluoride membrane (Immobirom-P®; Millipore Corp., Bedford, MA). Membranes were soaked in skim-milk in PBS, and biotinylated proteins were detected using streptavidin-peroxidase (VECTASTAIN® Elite ABC kit; Vector Laboratories Inc., Burlingame, CA) and ECL® system (Amersham International, Buckinghamshire, England).

Results and Discussion

The expression levels of TCR on thymocytes and peripheral T cells were significantly reduced in $\zeta T/\zeta T$ mice (20).

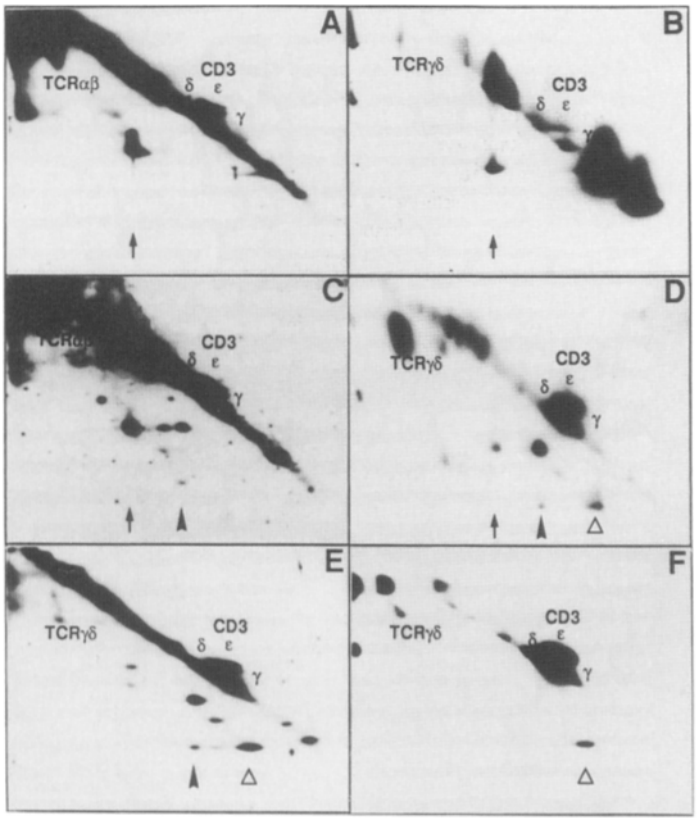

Figure 2. Biochemical analysis of the surface TCR composition. (*A*) Total and (*B*) DN-enriched thymocytes from wild type mice, i-IEL from (*C* and *D*) C57BL/6 (+/+) mice, (*E*) KSN *nu/nu* mice, and (*F*) ζT/ζT mice were surface biotinylated, lysed, and the TCR complex was immunoprecipitated with (*A* and *C*) anti-TCR-β mAB (H57-597), or *B*, *D*, *E*, and *F*) anti-TCR-δ mAb (3A10). Immunoprecipitates were separated by two-dimensional nonreducing-reducing SDS-PAGE, transferred onto PVDF membrane, and biotinylated proteins were detected as described in Materials and Methods. Positions of ζ–ζ (↑), ζ–FcRγ (▲), and FcRγ–FcRγ (△) dimers are indicated. Note that heterodimers of p12 (35, and Kuwabara, I., and Saito, T., manuscript in preparation) with ζ and FcRγ are observed in some panels. In three experiments (including *E*), densitometric analysis showed that the mean of the FcRγ of ζ–FcRγ dimers/FcRγ of FcRγ–FcRγ dimers is 1:11.9. Considering that the molar ratio of ζ and FcRγ in ζ–FcRγ dimers is 1, the expression level of FcRγ molecules is 13 times more than that of ζ.

This was observed in both α/β^+ and γ/δ^+ thymocytes as well as splenic T cells (Fig. 1 *A*). These data indicate that those cells predominantly express ζ as a component of the TCR complex, and extended to normal T cells the previous observation on in vitro cell lines that the expression of ζ is critical for a normal level of surface TCR expression (7–10).

When we analyzed i-IEL, however, a striking difference was observed (Fig. 1 *A*) (20). Similar to thymocytes and peripheral T cells, surface TCR expression of α/β^+ i-IEL was impaired by the ζT mutation. By contrast, surface TCR level of γ/δ^+ i-IEL from ζT/ζT mice was comparable with that of γ/δ^+ i-IEL from wild type mice. In γ/δ^+ i-IEL, two distinct subpopulations are known; the major population of CD8αα-bearing cells and the minor DN cells. The former is thought to differentiate extrathymically, whereas the latter is believed to derive from the thymus (29). Whether TCR expression of both γ/δ^+ i-IEL populations was resistant to the ζT mutation was analyzed by three-color flow cytometry. As shown in Fig. 1 *B*, the expression level of surface TCR in CD8αα$^+$ γ/δ^+ i-IEL from ζT/ζT mice remained normal, but that in DN γ/δ^+ i-IEL was reduced. These observations suggest that, in CD8αα$^+$ γ/δ^+ i-IEL, ζ family molecules other than ζ were predominantly associated with TCR.

To test this possibility, various preparations of T cells were analyzed for their composition of TCR complexes by a sensitive surface biotinylation (28) and two-dimensional nonreducing-reducing SDS-PAGE. TCR on α/β^+ and γ/δ^+ thymocytes as well as α/β^+ i-IEL from wild type mice was predominantly associated with ζ–ζ homodimers (Fig. 2, *A–C*). By contrast, analysis of TCR complexes on γ/δ^+ i-IEL revealed that, on these cells, both ζ and FcRγ contribute for TCR constitution (Fig. 2 *D*). Identification of ζ and FcRγ was confirmed by direct precipitation of these molecules with anti-ζ mAb (H146.968A) and anti-FcRγ antiserum, respectively (data not shown). Existence of two subpopulations in γ/δ^+ i-IEL from normal mice as mentioned above may complicate the analysis. To avoid this complexity, we utilized γ/δ^+ i-IEL from nude mice, which consist exclusively of CD8αα$^+$ cells (Ohno, H., unpublished observation). The result was more striking; these cells predominantly utilize FcRγ as TCR subunits, and ζ homodimers were hardly seen (Fig. 2 *E*). Assuming that the labeling efficiency of FcRγ is comparable between FcRγ homodimers and FcRγ–ζ heterodimers, these cells express 13 times more FcRγ molecules than ζ in TCR (see legend to Fig. 2). It is likely that DN γ/δ^+ i-IEL, like γ/δ^+ thymocytes, predominantly utilize ζ based on the following reasons: the amount of precipitated ζ from γ/δ^+ i-IEL from normal mice was much higher than that from nude mice; and TCR expression in DN γ/δ^+ i-IEL from ζT/ζT mice was reduced. We further examined whether the predominance of FcRγ in CD8αα$^+$ γ/δ^+ i-IEL is also observed in ζT/ζT mice. Fig. 2 *F* shows that this was the case. Collectively, these data indicate that the normal level of TCR expression in CD8αα$^+$ γ/δ^+ i-IEL from ζT/ζT mice correlates the predominant usage of FcRγ by TCR in this subset.

The preferential usage of FcRγ as a TCR component in

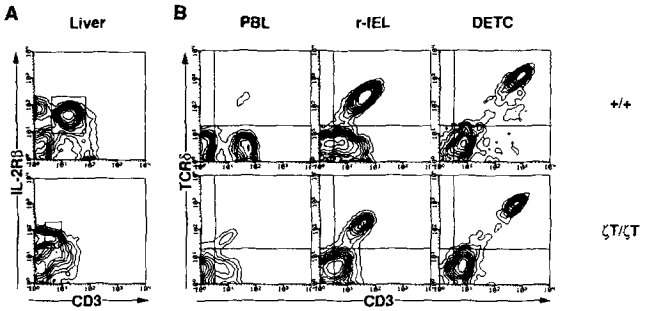

Figure 3. Expression of TCR on hepatic lymphocytes, r-IEL, and DETC from ζT/ζT mice. (*A*) CD3ε and IL-2Rβ expression on hepatic lymphocytes was analyzed as described in Materials and Methods. $CD3^{int}IL\text{-}2R\beta^{hi}$ cells, which are thought to develop extrathymically in the liver (30), are indicated with squares. (*B*) CD3ε and TCRδ expression on peripheral blood cells, r-IEL, and DETC were examined as described in Materials and Methods.

$CD8\alpha\alpha^+$ TCR γ/δ^+ i-IEL propelled us to analyze other T cell subsets for their usage of FcRγ. For this purpose, we examined the expression levels of TCR in T cells from ζT/ζT mice and compared them with those in T cells from wild type mice. Since η is far less efficient in assembly and surface expression of TCR compared with ζ (20, 22), it is reasonable to assume that T cells whose TCR expression is resistant to the ζT mutation utilize FcRγ as a component of the TCR complex. Similar to thymocytes and peripheral T cells, TCR on $CD3^{int}IL\text{-}2R\beta^{hi}$ hepatic lymphocytes (30) were reduced in ζT/ζT mice (Fig. 3 *A*). By contrast, surface TCR expression of r-IEL and DETC from ζT/ζT mice remained higher than that of peripheral T cells, indicating the predominant usage of FcRγ in these cells (Fig. 3 *B*).

The present study demonstrates that in mice, certain subsets of T cells preferentially utilize FcRγ, of ζ family molecules, as a component of the TCR complex. These include $CD8\alpha\alpha^+$ γ/δ^+ i-IEL, r-IEL, and DETC. It is of note that all these T cells express the TCR-γ/δ dimer and are localized in epithelia. In addition, these cells express a higher level of TCR compared with peripheral T cells. These three subsets of γ/δ^+ T cells are thought to be phylogenically old and to play a part in the surveillance of body surfaces that are exposed to the environment (26, 31, 32). In this respect, it is of particular interest that these T cells commonly utilize FcRγ as a component of their TCR complexes. FcRγ has one signaling motif in the cytoplasmic portion, whereas ζ has three motifs, which are supposed to be generated by intramolecular duplication. Furthermore, FcRγ is also expressed in NK cells, which are proposed to be the ancestor of T cells (33). Taken together, FcRγ may be a prototype of ζ family molecules.

Differential usage of ζ and FcRγ by distinct T cell subsets likely represents differences in lineage and/or function of these cells. Recently, evidence supporting the idea that TCR with FcRγ is functionally distinct from TCR with ζ is increasing. First, in vitro kinase assay on immunoprecipitates of TCR complexes containing FcRγ displayed a distinct phosphorylation pattern from that of TCR complexes containing ζ in T hybridoma cells (17). Second, the cytoplasmic tail of ζ but not FcRγ can transduce signals mediated through Thy-1 molecules (18). Third, ζ is replaced by FcRγ in T cells from tumor-bearing mice as well as cancer patients, and the change is accompanied with immune incompetence including an impaired antitumor response (19). Concerning the functional difference between distinct T cell subsets, an interesting result was reported in mucosal immunity (34); γ/δ^+ i-IEL from mice orally immunized with antigen abrogates tolerance induced in recipient mice by oral administration of the same antigen when transferred, while α/β^+ i-IEL provides helper function for antibody production in vitro. These functional differences may reflect differential usage of ζ family molecules by γ/δ^+ and α/β^+ i-IEL. Further study should shed light on the physiological role of T cell subsets bearing TCR with different ζ family molecules.

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Note Added in Proof: After submitting this paper, Malissen et al. (36) reported a similar observation that TCR complexes of i-IEL contain FcRγ homodimers.

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