T Cell Development in Mice Lacking All T Cell Receptor ζ Family Members (ζ, η, and FceRIγ)

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Summary

The ζ family includes ζ, η, and FceRIγ (Fcγ). Dimers of the ζ family proteins function as signal transducing subunits of the T cell antigen receptor (TCR), the pre-TCR, and a subset of Fc receptors. In mice lacking ζ/η chains, T cell development is impaired, yet low numbers of CD4+ and CD8+ T cells develop. This finding suggests either that pre-TCR and TCR complexes lacking a ζ family dimer can promote T cell maturation, or that in the absence of ζ/η, Fcγ serves as a subunit in TCR complexes. To elucidate the role of ζ family dimers in T cell development, we generated mice lacking expression of all of these proteins and compared their phenotype to mice lacking only ζ or Fcγ. The data reveal that surface complexes that are expressed in the absence of ζ family dimers are capable of transducing signals required for α/β-T cell development. Strikingly, T cells generated in both ζ/η- and ζ/η-/-Fcγ-/- mice exhibit a memory phenotype and elaborate interferon γ. Finally, examination of different T cell populations reveals that ζ/η and Fcγ have distinct expression patterns that correlate with their thymus dependency. A possible function for the differential expression of ζ family proteins may be to impart distinctive signaling properties to TCR complexes expressed on specific T cell populations.

The T cell antigen receptor (TCR) is a multimeric complex consisting of subunits that function primarily either in antigen recognition (α/β or γ/δ) or signal transduction (CD3-γ, -δ, and -ε, and a ζ family dimer) (1). The ζ family members constitute a group of structurally and functionally related proteins that include ζ, η (an alternatively spliced form of ζ), and FceRIγ (Fcγ; references 1, 2).

Thymocytes from mice lacking expression of both ζ and η chain (ζ/η-/-) are reduced in number and express extremely low levels of surface TCR relative to ζ/η+/+ mice (3-5). Nevertheless, T cell development is not completely arrested in ζ/η-/- mice as they contain both CD4+CD8+, or double positive (DP),1 and CD4+CD8- and CD4-CD8+, or single positive (SP) thymocytes and peripheral SP T cells (3-5). In contrast, expression of the CD3 (γ/δ/ε) complex is absolutely required for thymocyte development, as mice lacking expression of CD3-ε subunits fail to develop beyond the most immature CD4+CD8+, or double negative (DN), stage (6). The transition of DN thymocytes into DP thymocytes is regulated by the pre-TCR, a signaling complex composed of β chain, pre-Tα, and CD3 subunits, and which is also thought to include a ζ family dimer (7). The fact that low numbers of DP thymocytes (10-30% of normal) are generated in ζ/η-/- mice indicates that, though important, ζ and η are not essential for pre-TCR function. Likewise, the presence of low numbers of SP thymocytes and peripheral T cells in ζ/η-/- mice (3-5) demonstrates that ζ/η chains are not absolutely required for α/β-TCR expression or for promoting T cell development. Because of the extremely low levels of surface expression in ζ/η-/- mice, the subunit composition of surface pre-TCR and TCR complexes has not been accurately determined. One possibility is that the pre-TCR and TCR can be expressed in the absence of a ζ family dimer, and function, albeit inefficiently, to promote thymocyte development. Another possibility is that in ζ/η-/- mice the pre-TCR and/or α/β-TCR complexes associate with

1Abbreviations used in this paper: DETC, dendritic epidermal T cell; DN, double negative; DP, double positive; Fcy, FcεRIγ; FCM, flow cytometric analysis; I-IEL, intestinal intraepithelial lymphocytes; ITAM, immune-receptor tyrosine-based activation motif; SP, single positive.
Fcγ chain homodimers, since Fcγ is reported to be expressed during early thymocyte development (8–10). In mice lacking Fcγ, thymocyte development is unaffected, and therefore Fcγ normally does not play a significant role in the development of thymus-dependent T cells (11). Nevertheless, Fcγ, together with ζ chain, functions as a component of the TCR complex expressed on restricted populations of T cells ("thymus-independent" T cells), and in both Fcγ−/− and ζ−/− mice these T cells express relatively high levels of surface TCR (4, 5, 10). In addition, overexpression of Fcγ chain (or ζ chain) in thymocytes restores TCR surface expression and α/β-T cell development in ζ−/− mice (12–14). Therefore, all of the ζ family proteins are capable of independently supporting α/β-TCR surface expression and promoting the development of α/β-TCR+ thymocytes.

In this study, we have generated mice lacking all three ζ family proteins (ζ−/−, Fcγ−/− mice) and compared the T cell populations present in these animals to those found in mice lacking either ζ or Fcγ alone. The results provide direct evidence that pre-TCR and α/β-TCR complexes lacking a ζ family dimer are capable of supporting T cell development, positive selection, and T cell activation. Moreover, they reveal that, in the absence of specific stimuli, Fcγ is not normally expressed in thymus-dependent T cell populations, whereas both ζ and Fcγ are expressed in thymus-independent T cells. A possible function for the restricted expression of different ζ family proteins may be to modify the TCR signaling response in distinct populations of T cells.

Materials and Methods

Mice. The generation of ζ−/− and Fcγ−/− mice has been previously described (3, 11). ζ−/− (Fcγ−/−) mice were mated to ζ−/−/− Fcγ−/−/− mice and F1 progeny from these matings (i.e., ζ−/−/−−/−− Fcγ−/−/−/−) were then mated. Since the ζ−/− and Fcγ loci map to the same region of mouse chromosome 1 (2), F2 progeny were screened for crossover events that resulted in either a ζ−/−/−−/−− Fcγ−/−/−/− or ζ−/−/−−/−− Fcγ−/−/−/− genotype. One ζ−/−/−−/−− Fcγ−/−/−/− mouse, identified among the first 100 F2 progeny analyzed, served as a founder line for the generation of ζ−/−/−−/−− Fcγ−/−/−/− mice. Genotypes were identified initially by Southern blotting as previously described (3, 11) and were subsequently screened by PCR. Screening of ζ−/− was performed with oligonucleotides Z1: 5′-GAGAGAGGAAATGACGTCTTGGAGAAGA-3′; Z2: 5′-AAGAGCGATCTGAGTATGAG-3′; and ZNEO: 5′-670 T Cell Development in the Absence of TCR-ζ Family Dimers 1994 T Cell Development in the Absence of TCR-ζ Family Dimers 1994 T Cell Development in the Absence of TCR-ζ Family Dimers 1994 T Cell Development in the Absence of TCR-ζ Family Dimers 1994 T Cell Development in the Absence of TCR-ζ Family Dimers 1994 T Cell Development in the Absence of TCR-ζ Family Dimers 1994 T Cell Development in the Absence of TCR-ζ Family Dimers
described in Materials and Methods. Examination of thymocytes from $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice by FCM revealed a phenotype essentially identical to that of $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice (Fig. 1 A). In addition, $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice contained similar total numbers of thymocytes (10-30% of normal, data not shown), which consisted almost entirely of DN and DP cells. DN thymocytes from $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice express extremely low but detectable levels of TCR as assessed by staining with anti-CD3 $\zeta$ and anti-TCR $\beta$ mAbs (3, 14). A similarly low but discernable level of TCR surface expression was observed on DP thymocytes from $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice by FCM (Fig. 1 A). Significantly, although CD4$^{+}$CD8$^{-}$ and CD4$^{-}$CD8$^{+}$ SP cells were not readily detectable in the thymus, SP T cells were present in the lymph nodes (data not shown) and spleen (Fig. 1 B) of $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice in numbers similar to those observed in $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice (data not shown). Together, these findings demonstrate that the low but detectable level of TCR expression on DP thymocytes from $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice implies a function for $\zeta$ (and/or $\gamma$) as components of the pre-TCR. However, the fact that some DP thymocytes are generated in both $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ and $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice indicates that the pre-TCR is capable, albeit inefficiently, of transducing signals that promote the development of DN thymocytes to the DP stage in the absence of all $\zeta$ family proteins. To further evaluate the ability of surface complexes expressed on DN thymocytes in $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice to transduce signals that promote the formation of DP thymocytes, we generated $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}} - RAG-1^{-/2}$ mice. TCR-deficient mice express barely detectable levels of surface TCR, and in RAG-1$^{-}$, $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice resulted in increased thymic cellularity (5-20× control) and the generation of large numbers of DP thymocytes. Together these data demonstrate that both early and late stages of thymocyte development are not absolutely dependent on the expression of $\zeta$ family proteins.

Tryptophan has been shown to be severely compromised at the DN stage in $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice (17). Whereas the most mature subset of DN thymocytes (CD4$^{+}$CD8$^{-}$) constitutes 10-20% of the DN population in normal adults and in mice lacking only Fcy (Fig. 1 C), these cells are nearly absent in $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice (reference 17 and Fig. 1 C). Examination of DN thymocyte subsets from both $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ and $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice revealed a block at the identical (CD4$^{+}$/CD25$^{+}$) stage of maturation (Fig. 1 C), indicating that neither Fcy nor $\zeta$ chain is required for thymocyte development before the CD4$^{+}$/CD25$^{+}$ stage. Since the generation and subsequent expansion of CD4$^{+}$CD25$^{-}$ DN thymocytes is thought to be controlled by signaling through the pre-TCR complex (7, 18), the paucity of CD4$^{+}$CD25$^{-}$ DN thymocytes in $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice implies a function for $\zeta$ (and/or $\gamma$) as components of the pre-TCR. However, the fact that some DP thymocytes are generated in both $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ and $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice indicates that the pre-TCR is capable, albeit inefficiently, of transducing signals that promote the development of DN thymocytes to the DP stage in the absence of all $\zeta$ family proteins. To further evaluate the ability of surface complexes expressed on DN thymocytes in $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice to transduce signals that promote the formation of DP thymocytes, we generated $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}} - RAG-1^{-/2}$ mice. TCR-deficient mice express barely detectable levels of surface TCR, and in RAG-1$^{-/2}$, $\zeta^\gamma^{-} - FcY^{-}^{\alpha^{-}}$ mice resulted in increased thymic cellularity (5-20× control) and the generation of large numbers of DP thymocytes. Together these data demonstrate that both early and late stages of thymocyte development are not absolutely dependent on the expression of $\zeta$ family proteins.
cells from ζ/η−/−Fcγ−/− mice were also capable of transducing activating signals, we examined lymph node T cells for expression of cell surface molecules associated with activation and memory. Surprisingly, although T cells from ζ/η−/−Fcγ−/− mice express extremely low levels of surface TCR, a high percentage of these cells appeared to have an activated or memory phenotype (i.e., CD44h, CD62Lh; Fig. 3). A high percentage of SP T cells from ζ/η−/− were also CD44h, CD62Lh, whereas the majority of T cells from both control (ζ/η+/+/Fcγ−/+) and Fcγ−/− mice displayed a naïve phenotype (CD44h, CD62Lh; Fig. 3). Nevertheless, T cells from both ζ/η−/−Fcγ−/− and ζ/η−/− mice were largely refractory to direct TCR stimulation in vitro as they did not appreciably increase levels of CD69 or IL-2Rα and proliferated poorly in response to treatment with cross-linking anti-TCR antibodies or anti-TCR plus anti-CD28 (data not shown).

We next examined the ability of T cells from ζ/η−/− and ζ/η−/−Fcγ−/− mice to produce cytokines after stimulation for 18 h with either PMA and ionomycin or anti-CD3ε mAb. Stimulation of purified T cells from ζ/η−/− and ζ/η−/−Fcγ−/− mice (as well as from Fcγ−/− and ζ/η+/+/Fcγ−/+) resulted in production of IL-2 (Table 1), but in all samples IL-4 and IL-10 remained undetectable (data not shown). However, although T cells from ζ/η−/−Fcγ−/− and Fcγ−/− mice produced only low levels of IFN-γ after stimulation, T cells from both ζ/η−/− and ζ/η−/−Fcγ−/− mice produced large quantities of IFN-γ after stimulation (Table 1). A similar cytokine profile was observed when cytokine production was assessed by RT-PCR (Fig. 4). IFN-γ mRNA was also detectable in freshly isolated ex vivo (unstimulated) T cells from ζ/η−/−Fcγ−/− mice and ζ/η−/− mice by RT-PCR (Fig. 4). Since purified populations of T cells were used for these experiments, it is unlikely that IFN-γ was derived from contaminating cell populations, such as N K cells. Together, these findings indicate that despite their low levels of surface TCR, a high percentage of T cells from both ζ/η−/− and ζ/η−/−Fcγ−/− mice appear to be endogenously activated and exhibit a Th1 memory cell phenotype.

**EX VIVO CD4+ SPLENOCYTES**

| CD44   | CD62L | CD5    | FSC    | CD2     |
|--------|-------|--------|--------|---------|
| ζ+/+  | ζ+/−  | ζ−/+  | ζ−/−  |
| Fcγ+/+| Fcγ/−| Fcγ+/+| Fcγ/−|

**Figure 3.** Surface phenotype of peripheral T cells from ζ/η−/−Fcγ−/− mice. Activation-memory phenotype of splenic T cells. Cells were stained with anti-CD4-PE versus anti-CD44-FITC, anti-CD62L-FITC, anti-CD2-FITC, or anti-CD5-FITC. Single color histograms show staining on CD4+ T cells. Data were collected on 5 × 10^6 gated cells. Shaded areas represent staining with experimental antibodies and solid lines depict staining with negative control antibodies.
indicates homozygosity for the mutant g allele, and CD4–CD8–TCRlo/A /B cells are virtually absent in Fc–/ mice (data not shown). These results are consistent with the idea that thymus-dependent i-IELs express g but not Fcγ during their development. On the other hand, thymus-independent i-IELs have been shown to express both g and Fcγ chains (8), and mice lacking either g or Fcγ contain a/B–TCR + CD8–/α+ and γδ–TCR + i-IELs that express only moderately reduced levels of surface TCR when compared with similar populations of i-IELs from Fc–/ mice (references 4, 5, and Fig. 5). Significantly, in the absence of g, n, and Fcγ chains, all population of i-IELs (thymus-dependent and -independent) are TCRg– (Fig. 5).

To examine the lineage of TCR– i-IELs in g–/ mice, cells were analyzed for the presence of intracellular TCR–/B and TCR–/δ chains. Interestingly, intracellular staining for TCR–/δ chains revealed that TCR–/δ lineage T cells are virtually absent in g–/–Fcy–/– mice whereas TCR–/α/β lineage T cells are readily detected in the same animals (Fig. 5, bottom). Notably, TCR–γδ cells are also markedly reduced in number in g–/– mice, but not Fcγ–/– mice, despite the fact that i-IELs from these animals express comparable levels of surface TCR (references 4, 5, and Fig. 5, 4th and 5th columns). Together, these findings indicate that either (a) the generation and/or survival of TCR–/γδ T cells is particularly dependent on expression of g–/ chain, or (b) Fcγ is poorly expressed in developing γδ lineage T cells. Finally, these results demonstrate that expression of a g family dimer is required for efficient TCR surface expression on all T cell populations including both thymus-dependent and thymus-independent TCR–/α/β+ and TCR–/γδ– cells.

γδ–TCR DETCs and NKL1+ T cells use g chain for TCR Surface Expression. We next examined mice lacking expression of g, Fcγ, or all g family proteins for the presence of DETCs that express γδ–TCR and are thymically derived (26). We observed that though present, DETCs from g–/– mice and γδ–Fcy–/– mice express extremely low or undetectable levels of surface TCR, whereas DETCs from Fcγ–/– mice express high levels of γδ–TCR (Fig. 6 A). These results were unexpected as it had been previously reported that g–/– mice contain DETCs that express relatively high levels of γδ–TCR (27–29).

We also examined thymocytes from g–/– and g–/– mice for the presence of NKL1+ T cells that are also thymically derived but not necessarily thymus-dependent (30). Although both g–/– and γδ–Fcy–/– mice contained thymocytes of the expected “activation-NK” phenotype (i.e., NKL1, IL-2R, CD44, MEL-14) TCR+ cells were detectable only in g–/– mice and these cells were exclusively γδ–/– mice (Fig. 6 B). Significantly, although an earlier study had reported the presence of large

| Table 1. Cytokine Elaboration (10^6 Cells) |
|------------------------------------------|
| Mouse | Stimuli | IFNγ | IL-2 |
|-------|--------|------|------|
|       | PM +1  | 79   | 3,228|
|       | anti-CD3| 31   | 22   |
|       | PM +1  | 3,403| 490  |
|       | anti-CD3| 1,596| 272  |
|       | PM +1  | 3,330| 1,562|
|       | anti-CD3| 1,552| 846  |
|       | PM +1  | 224  | 2,477|
|       | anti-CD3| 175  | 60   |

Results from a representative experiment are shown. Splenic T cells were purified and 10^6 cells were stimulated in culture for 18 h with PM +1 (10 ng/ml) and ionomycin (1 μM) (PM +1) or plate-bound anti-CD3–. Supernatants were serially diluted and tested by ELISA as described in Materials and Methods. Concentrations were determined by comparison with known concentrations of cytokines provided by the manufacturers of the monoclonal antibodies used for ELISA.
curs at two specific checkpoints is revealing as to the in vivo function of the ζ family dimers. Early DN thymocytes from ζ/η−/− mice can give rise to DP thymocytes; however, DP thymocytes are reduced in number as are their immediate precursors (CD4−CD8−/CD4−CD8−/CD25− thymocytes). Since Fcγ is reported to be expressed in early thymocytes (8–10), and because transgenic Fcγ can restore development of DN CD4−CD25− thymocytes in ζ/η−/− mice (13), it was possible that endogenous Fcγ enabled the development of some thymocytes in the absence of ζ/η by functioning as a component of the pre-TCR. However, the observation that ζ/η−/− and ζ/η−/−Fcy−/− mice contain similar numbers of DP thymocytes and show partial arrest at the same stage of development demonstrates that ζ family members are not absolutely required for pre-TCR function. Notwithstanding, the markedly reduced number of both DN CD24−CD4− mice and DP thymocytes in both ζ/η−/− and ζ/η−/−Fcy−/− mice argues that in the presence of a ζ family dimer, the pre-TCR is much more efficient at promoting this transition. The other partial developmental arrest observed in both ζ/η−/− and ζ/η−/−Fcy−/− occurs during the transition of cells from the DP to SP stage of development. SP thymocytes are nearly undetectable in ζ/η−/− and ζ/η−/−Fcy−/− mice, yet both mice contain significant numbers of peripheral SP T cells that accumulate with age. Thus ζ family proteins are also not absolutely required for late stages of T cell development. Nevertheless, ζ chain is required for efficient TCR surface expression and ζ immunoreceptor tyrosine-based activation motif (ITAM)-mediated signals have been shown to play an important role in positive and negative selection of the T cell repertoire (32).

Figure 5. i-IEL development in ζ/η−/−Fcy−/− mice. i-IELs were prepared from mice as described (13) and three-color FCM was performed. For internal staining, cells were first stained with anti-CD4 and anti-CD8β externally, then treated with intracellular staining buffer followed by staining with anti-CD3, anti-TCR-β, or anti-TCR-δ mAbs. Data depict two-color analysis of CD3 versus TCR-β or CD3 versus TCR-δ on software-gated CD4−CD8−β cells. Numbers reflect the percentage of gated CD4−CD8−β cells in that quadrant.

Discussion

The results of this study demonstrate that “partial” TCR complexes that lack ζ family proteins (ζ, η, and Fcγ) can promote the maturation of at least some thymocytes. Indeed, thymocytes from ζ/η−/−Fcy−/− mice appear to undergo a relatively normal developmental program; originating from precursor DN thymocytes they develop to the DP stage, undergo positive selection, and emerge as SP T cells. T cells generated in ζ/η−/−Fcy−/− mice express a functionally active TCR such that stimulation of these complexes by direct engagement results in the production of specific cytokines. Collectively, these observations indicate that pre-TCR and TCR complexes that contain CD3 subunits but not a ζ family dimer can transduce signals normally associated with fully assembled TCR complexes.

Although some thymocytes are capable of developing in ζ/η−/− and ζ/η−/−Fcy−/− mice, the partial arrest that occurs at two specific checkpoints is revealing as to the in vivo function of the ζ family dimers. Early DN thymocytes from ζ/η−/− mice can give rise to DP thymocytes; however, DP thymocytes are reduced in number as are their immediate precursors (CD4−CD8−/CD4−CD8−/CD25− thymocytes). Since Fcγ is reported to be expressed in early thymocytes (8–10), and because transgenic Fcγ can restore development of DN CD4−CD25− thymocytes in ζ/η−/− mice (13), it was possible that endogenous Fcγ enabled the development of some thymocytes in the absence of ζ/η by functioning as a component of the pre-TCR. However, the observation that ζ/η−/− and ζ/η−/−Fcy−/− mice contain similar numbers of DP thymocytes and show partial arrest at the same stage of development demonstrates that ζ family members are not absolutely required for pre-TCR function. Notwithstanding, the markedly reduced number of both DN CD24−CD4− mice and DP thymocytes in both ζ/η−/− and ζ/η−/−Fcy−/− mice argues that in the presence of a ζ family dimer, the pre-TCR is much more efficient at promoting this transition. The other partial developmental arrest observed in both ζ/η−/− and ζ/η−/−Fcy−/− occurs during the transition of cells from the DP to SP stage of development. SP thymocytes are nearly undetectable in ζ/η−/− and ζ/η−/−Fcy−/− mice, yet both mice contain significant numbers of peripheral SP T cells that accumulate with age. Thus ζ family proteins are also not absolutely required for late stages of T cell development. Nevertheless, ζ chain is required for efficient TCR surface expression and ζ immunoreceptor tyrosine-based activation motif (ITAM)-mediated signals have been shown to play an important role in positive and negative selection of the T cell repertoire (32).

T cell receptors expressed on mature T cells from ζ/η−/− mice exhibit high affinity for self-ligand, a finding not observed in TCRs derived from normal mice (33). According to signal strength models of selection, it is likely that only those T cells with relatively high affinity for self-ligands are positively selected and survive in ζ/η−/− and ζ/η−/−Fcy−/− mice. The absence of ζ-mediated signaling during thymic selection and the consequent selection of T cells with high affinity receptors for self has a critical impact on the phenotype and responsiveness of the mature T cells that are generated. Indeed, despite their low levels of surface TCR, a high percentage of T cells from both ζ/η−/− and ζ/η−/−Fcy−/− mice appear to be endogenously activated and exhibit a Th1 memory cell phenotype. The high-affinity TCRs expressed by these cells could contribute to this phenotype, as high affinity interactions, with a long association rate, could promote differentiation of cells towards Th1 type memory cells by enabling coreceptor molecules to be effectively recruited into the receptor complex. Indeed, recent studies have suggested that individual cytokine responses may be regulated in a hierarchical manner that depends on the particular signaling threshold and the recruitment of costimulatory molecules (34). Although these findings (34) are based on data obtained by varying the concentration of ligand, our results would suggest that both ligand density and TCR affinity influence the biochemical
response made by a particular T cell. Since T cells from \( \zeta \eta^-/- \) and \( \zeta \eta^-/-F_c^+/- \) mice express extremely low levels of TCR, the affinity of TCR-ligand interactions, rather than the absolute number of TCR complexes that are engaged, could be critical for dictating the type of cytokine response generated by T cells in our model. Alternatively, the skewing toward a Th1-like response in these mice may also be reflective of the genetic background (C57BL/6) or the functional alteration of NK1.1+ T cell populations that are known to produce IL-4 (28). Whatever the foundation for the Th1 phenotype, the generation of Th1 type cytokines appears to have important physiologic consequences for the Th1 phenotype, the generation of Th1 type cytokines, these observations suggest that expression of Fcγ can be induced by lymphokines. However, this property is not universally shared by all T cell populations, as peripheral CD4+ \( \alpha / \beta^-/- \) Thymocytes and CD8+ \( \alpha / \beta^-/- \) T cells do not induce synthesis of Fcγ after cytokine stimulation (reference 8 and our unpublished data).

The restricted expression of \( \zeta \eta^-/- \) and Fcγ in different T cell populations also suggests that these proteins may perform specific functions, perhaps by quantitatively or qualitatively influencing the TCR signaling response. \( \zeta \eta^-/- \) chain, which contains 3 ITAM signaling motifs appears optimized to facilitate the development of thymus-dependent, self-MHC-restricted T cells. Indeed, thymocyte positive selection is markedly impaired in transgenic mice in which Fcγ chain, which contains only a single ITAM, is substituted for the \( \zeta \eta^-/- \) chain (13). The ability of \( \zeta \eta^-/- \) chain to amplify signals resulting from low avidity TCR-ligand interactions is therefore particularly critical for thymocyte selection (32). On the other hand, the reduced signaling potential of Fcγ chain may be important for limiting the responsiveness of i-IELs and lymphokine-activated T cells to antigen. Thus, the restricted potential of different T cell populations to express and use specific members of the \( \zeta \eta^-/- \) family as components of the TCR could be a mechanism for regulating the T cell response to antigen.

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References

1. Klausner, R.D., J. Lippincott-Schwartz, and J.S. Bonifacino. 1990. The T cell antigen receptor. Annu. Rev. Cell Biol. 6: 403–431.

2. Küster, H., H. Thompson, and J.-P. Kinet. 1990. Characterization and expression of the gene for the human Fc receptor \(\gamma\) subunit. Definition of a new gene family. J. Biol. Chem. 265:6448–6452.

3. Love, P.E., E.W. Shores, M.D. Johnson, M. Tremblay, E.J. Lee, A. Grinberg, S.P. Huang, A. Singer, and H. Westphal. 1993. T cell development in mice that lack the \(\zeta\) chain of the T cell antigen receptor complex. Science. 261:918–921.

4. Malissen, M., A. Gillet, B. Rocha, J. Trucy, E. Vivier, C. Boyer, F. Kontgen, N. Brun, G. Maaza, E. Sapanpoulou, et al. 1993. T cell development in mice lacking the CD3-\(\zeta\)-\(\eta\) gene. EMBO (Eur. Mol. Biol. 0 rgan.) J. 12:4347–4355.

5. Liu, C.-P., R. Ueda, J. She, J. Sancho, B. Warg, G. Weddell, J. Loring, C. Kurahara, E.C. Dudley, A. Hayday, et al. 1993. Abnormal T cell development in CD3-\(\zeta\)-deficient mutant mice and identification of a novel T cell population in the intestine. EMBO (Eur. Mol. Biol. Organ.) J. 12:4863–4875.

6. Malissen, M., A. Gillet, L. Ardaun, G. Bouvier, J. Trucy, P. Ferrier, E. Vivier, and B. Malissen. 1995. Altered T cell development in mice with a targeted mutation of the CD3\(\zeta\) gene. EMBO (Eur. Mol. Biol. Organ.) J. 14:4641–4653.

7. von Boehmer, H., and H.J. Fehling. 1997. Structure and function of the pre-T cell receptor. Annu. Rev. Immunol. 15: 432–452.

8. Guy-Grand, D., B. Rocha, P. Mintz, M. Malaisse-Seris, F. Selz, B. Malissen, and P. Vassalli. 1994. Different use of T cell receptor transducing modules in two populations of gut in- nertinal lymphocytes are related to distinct pathways of T cell differentiation. J. Exp. Med. 180:673–679.

9. Flamand, V., E.W. Shores, T. Tran, K. Huang, E. Lee, A. Grinberg, J.-P. Kinet, and P.E. Love. 1996. Delayed maturation of CD4-CD8- FcReII/III+ T and NK cell precursors in FcR\(\gamma\) transgenic mice. J. Exp. Med. 184:1725–1735.

10. Heikin, H., R.-J. Schulz, J.V. Ravetch, E. Reinherz, and S. Koyasu. 1996. T lymphocyte development in the absence of FcR\(\gamma\) subunit: analysis of thymus-dependent and independent \(\alpha\beta\) and \(\gamma\delta\) pathways. Eur. J. Immunol. 26:1935–1943.

11. Takai, T., M. Li, D. Sylvestre, R. Clynés, and J.V. Ravetch. 1994. FcR\(\gamma\) chain deletion results in pleiotropic effector cell defects. Cell. 76: 519–529.

12. Liu, C.-P., W.-J. Lin, M. Huang, J.W. Kappler, and P. Marrack. 1997. Development and function of T cells in T cell antigen receptor/CD3\(\zeta\) knockout mice reconstituted with FcR\(\gamma\)cy. Proc. Natl. Acad. Sci. USA. 94:616–621.

13. Shores, E., V. Flamand, T. Tran, A. Grinberg, J.-P. Kinet, and P.E. Love. 1997. FcR\(\gamma\)cy can support T cell development and function and promote survival in mice lacking endogenous TCR \(\zeta\) chain. J. Immunol. 159:222–230.

14. Shores, E.W., K. Huang, T. Tran, E. Lee, A. Grinberg, and P.E. Love. 1994. Role of TCR-\(\zeta\) chain in T cell development and selection. Science. 266:1047–1050.

15. Borkowski, T.A., J.J. Letterio, A.G. Farr, and M. C. Udey. 1996. A role for endogenous transforming growth factor \(\beta1\) in Langerhans cell biology: the skin of transforming growth factor \(\beta1\) null mice is devoid of epidermal Langerhans cells. J. Exp. Med. 184:2417–2422.

16. Nakayama, T., A. Singer, E.D. Hsi, and L.E. Samelson. 1989. Intrathymic signalling in immature CD4+CD8+ thymocytes results in tyrosine phosphorylation of the T cell receptor zeta chain. Nature. 341:651–654.

17. Crompton, T., M. Mmoire, H.R. Macdonald, and B. Malissen. 1994. Double-negative thymocyte subset in CD3\(\gamma\) chain-deficient mice: absence of HSA+CD44+CD25+ cells. Eur. J. Immunol. 24:1901–1907.

18. Godfrey, D.I., and A. Zlotnik. 1993. Control points in early T cell development. Immunol. Today. 14:547–553.

19. Leveil, C.N., P. Mombaerts, A. Iglesias, S. Tonegawa, and K. Eichmann. 1993. Restoration of early thymocyte differentiation in T cell receptor beta-chain-deficient mutant mice by transmembrane signaling through CD3-\(\epsilon\). Proc. Natl. Acad. Sci. USA. 90:11401–11405.

20. Leveil, C.N., P. Mombaerts, B. Warg, H. Kohler, S. Tonegawa, K. Eichmann, and C. Terhorst. 1995. Regulation of thymocyte development through CD3 functional association between p56lck and CD3 gamma in early thymic selection. Immunity. 3:215–222.

21. Robey, E., and B.J. Fowlkes. 1994. Selective events in T cell development. Annu. Rev. Immunol. 12:675–705.

22. Guidos, C.J. 1996. Positive selection of CD4+ and CD8+ T cells. Curr. Opin. Immunol. 8:225–232.

23. Lefrançois, L. 1991. Phenotypic complexity of intraepithelial lymphocytes of the small intestine. J. Immunol. 147:1746–1751.

24. Rocha, B., P. Vassalli and D. Guy-Grand. 1994. Thymic and extrathymic origins of gut intraepithelial lymphocyte populations in mice. J. Exp. Med. 180:681–686.

25. Simpson, S., G. Holländer, J. She, C. Leveil, M. Huang, and C. Terhorst. 1995. Selection of peripheral and intestinal T lymphocytes lacking CD3. Int. Immunol. 7:287–293.

26. Allison, J.P., and W. Haraun. 1991. The immunobiology of T cells with invariant \(\gamma\delta\) antigen receptors. Annu. Rev. Immunol. 9:679–705.

27. Shimada, S. 1994. T cell receptor expression by dendritic epidermal T cells. J. Dermatol. (Tokyo). 182:633–638.

28. Ohno, H., T. Aoe, S. Taki, D. Kitamura, Y. Ishida, K. Rajewsky, and T. Saito. 1993. Development and functional impairment of T cells in mice lacking CD3\(\zeta\) chains. EMBO (Eur. Mol. Biol. Organ.) J. 12:4357–4366.

29. Ohno, H., S. Ono, N. Hirayama, S. Shimada, and T. Saito. 1994. Preferential usage of the Fc receptor complex by \(\gamma\delta\) T cells localized in epithelium. J. Exp. Med. 179:365–369.

30. Macdonald, H.R. 1995. N.K.1.1+ T cell receptor-\(\alpha\beta\) cells new clues to their origin, specificity, and function. J. Exp. Med. 182:633–638.

31. Arase, H., S. Ono, N. Arase, S.Y. Park, K. Wakioka, H. Watanabe, H. Ohno, and T. Saito. 1995. Developmental arrest of N.K.1.1+ T cell antigen receptor (TCR-\(\alpha\beta\))+ T cells and expansion of N.K.1.1+ TCR-\(\gamma\delta\) T cell development in CD3\(\zeta\)-deficient mice. J. Exp. Med. 182:891–895.
32. Shores, E.W., T. Tran, A. Grinberg, C.L. Sommers, H. Shen, and P.E. Love. 1997. Role of the multiple T cell receptor (TCR)-ζ chain signaling motifs in selection of the T cell repertoire. J. Exp. Med. 185:893–900.

33. Lin, S.-Y., L. Ardouin, A. Gillet, M. Malissen, and B. Malissen. 1997. The single positive T cells found in CD3-ζ/η-/- mice overtly react with self-major histocompatibility complex molecules upon restoration of normal surface density of T cell receptor-CD3 complex. J. Exp. Med. 185:707–715.

34. Itoh, Y., and R.N. Germain. 1997. Single cell analysis reveals regulated hierarchical T cell antigen receptor signaling thresholds and intraclass heterogeneity for individual cytokine responses of CD4+ T cells. J. Exp. Med. 186:757–766.

35. Curnow, S.J., C. Boyer, M. Buferne, and A.-M. Schmitt-Verhulst. 1995. TCR-associated ζ-FcγRIγ heterodimers on CD4−CD8−NK1.1+ T cells selected by specific class I MHC antigen. Immunity 3:427–438.

36. Koyasu, S., L. D’Adamio, A.R.N. Arulanandam, S. Abraham, L.K. Clayton, and E.L. Reinherz. 1992. T cell receptor complexes containing FcγRIγ heterodimers in lieu of CD3ζ and CD3η components: a novel isoform expressed in large granular lymphocytes. J. Exp. Med. 175:203–209.

37. Qian, D., A.I. Sperling, D.W. Lacenic, Y. Tatsumi, T.A. Barrett, J.A. Bluestone, and F.W. Fitch. 1993. The gamma chain of the high-affinity receptor for IgE is a major functional subunit of the T-cell antigen receptor complex in gamma delta lymphocytes. Proc. Natl. Acad. Sci. USA. 90:11875–11879.