

Differentiation of IFN γ -producing CD4⁺ T cells deficient in ζ chain expression

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Expression of T cell receptor (TCR) on T cells and its signaling through TCRs are important factors of CD4⁺ T helper cell (Th) differentiation. In the present study, we have investigated the differentiation of IFN γ -producing cells from mice that lack the expression of a key TCR signaling molecule, ζ chain (ζ KO mice). The CD4⁺ T cells isolated from ζ KO mice, ζ KO mice expressing an FcR γ chain transgene (FcR γ TG, ζ KO mice), and C57BL/6 mice were stimulated with the anti-TCR antibody, H57, in a culture condition that favors the Th1 cell differentiation. The results from intracellular cytokine staining (ICS) studies showed that T cells from C57BL/6 mice had the lowest percentage of IFN γ -producing CD4⁺ T cells (IFN γ ⁺CD4⁺ T cells), and more of the CD4⁺ T cells from ζ KO mice produced IFN γ than do T cells from FcR γ TG, ζ KO mice. The results from ELISA studies were consistent with the ICS studies. These results suggest that expression of the ζ chain, and to a lesser extent, expression of the FcR γ TG chain may decrease the differentiation of CD4⁺ T cells into IFN γ -producing cells. Interestingly, further ICS studies showed that some of the CD4⁺ T cells cultured under the same conditions also produced IL-10 (IL10⁺CD4⁺ T cells), and more of the CD4⁺ T cells from ζ KO mice than from C57BL/6 mice produced intracellular IL10. The FcR γ TG, ζ KO mice also showed more IL10⁺CD4⁺ T cells than that of C57BL/6 mice, but the percentage of such cells was less than that of T cells from ζ KO mice. Double staining of T cells from these three strains of mice showed that >50% of the IFN γ ⁺CD4⁺ T cells from ζ KO mice or FcR γ TG, ζ KO mice also co-produced IL10. However, the number of ζ KO mouse T cells that produced only IFN γ (IFN γ ⁺/IL10⁻ single producers) and cells that co-produced both IFN γ /IL10 (IFN γ ⁺/IL10⁺ co-producers) was significantly higher than those in FcR γ TG, ζ KO mice. On the other hand, less than 25% of IFN γ ⁺CD4⁺ T cells from C57BL/6 mice co-produced IL10. Although CD4⁺ T cells from ζ KO mice have the highest percentage of IFN γ ⁺/IL10⁺ co-producers as demonstrated in ICS studies following *in vitro* culture, ELISA studies showed that their CD4⁺ T cells secreted much less IL10 when compared to T cells from C57BL/6 mice and FcR γ TG, ζ KO mice. It has been shown that IL18 may play an important role in the differentiation of IFN γ -producing T cells. To determine the role of IL18 in our studies, we found that the addition of IL18 to the cell cultures resulted in a significant increase of total IFN γ ⁺CD4⁺ T cells derived from C57BL/6 and FcR γ TG, ζ KO mice after activation through TCR. However, the presence of IL18 did not significantly change the percentage of IFN γ ⁺CD4⁺ T cells from ζ KO mice. On the other hand, the addition of IL18 resulted in a significant decrease of IL10⁺CD4⁺ T cells in ζ KO mice and FcR γ TG, ζ KO mice but not in C57BL/6 mice. Further studies demonstrated that the presence of IL18 increased IFN γ ⁺/IL10⁻ single producers of CD4⁺ T cells from all three strains of mice, whereas it decreased IFN γ ⁺/IL10⁺ co-producers from ζ KO mice and FcR γ TG, ζ KO mice but not from C57BL/6 mice. In summary, these results suggest that ζ chain-deficient T cells and not normal ζ chain bearing T cells differentiate more easily into IFN γ -producing T cells, whether or not they co-produce IL10. Therefore, expression of the ζ chain in TCR complexes may result in an inhibitory effect on T cell differentiation into IFN γ -producing Th1 cells. Moreover, IL18 has a distinct effect on the differentiation of IFN γ ⁺/IL10⁻ single producers and IFN γ ⁺/IL10⁺ co-producers in these animals.