

A Novel System to Characterize Murine Recent Thymic Emigrants

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To analyze the surface antigen phenotype and the functional capacity of recent thymic emigrants (RTEs) in unmanipulated mice, we used mice transgenic for enhanced green fluorescent protein (eGFP) under the control of the RAG2 promoter. As predicted, thymocytes from RAG2pGFP transgenic mice begin to express GFP at the late CD4^{lo}CD8^{lo} stage, when the recombinase is first expressed. However, the GFP protein lacks the cell cycle-dependent degradation signal carried by the RAG2 protein, and the GFP signal lingers in these cells (with a 16-18h half-life) through the mature CD4⁺CD8^{lo} and CD4^{lo}CD8⁺ (SP) compartments, well after RAG2 expression is extinguished. A population of peripheral T cells is GFP^{hi}, and these cells can be classified as RTEs because they disappear within 2 weeks of adult thymectomy. Therefore, analyzing GFP^{hi} peripheral T cells in RAG2pGFP transgenic mice offers a means of characterizing RTEs in unmanipulated mice. RTEs make up a larger fraction of the peripheral T cell pool in young animals and are characterized by a higher CD4:CD8 ratio than is typical of the rest of the peripheral T cell pool. Relative to their SP thymocyte counterparts, RTEs downregulate HSA and CD69 expression and upregulate Qa2, Ly6C, CD45RB, and IL7R α expression and gradually increase in size as they lose GFP expression. Thus, T cells continue maturation at the level of surface antigen phenotype in the lymphoid periphery. This phenotypic maturation is mirrored by the acquisition of proliferative capacity. Sorted GFP⁺CD4⁺ splenic T cells proliferate poorly in response to anti-CD3 + anti-CD28 stimulation relative to GFP^{lo}CD4⁺ splenic T cells, although both populations upregulate CD69 and CD25 expression to the same extent and with similar kinetics. The addition of IL-2 overcomes the proliferative defect of GFP⁺CD4⁺ splenic T cells. These data suggest that in addition to completing their sequence of phenotypic maturation, CD4⁺ T cells acquire complete immunocompetence only after they reach the lymphoid periphery. We are currently extending our studies to quantitate the proliferative and cytolytic capacity of CD8⁺ RTEs.