

Mechanism of Third Signal Cytokines, IL-12 and IFN- α/β , in Programming CTL Differentiation

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Antigen (signal 1) and costimulation (signal 2) stimulate proliferation of CD8 T lymphocytes, but a third signal in the form of IL-12 or IFN- α is required for CTL differentiation leading to acquisition of effector functions and memory. In the absence of a third signal Ag recognition gives rise to a non-lytic population and those cells that persist long-term in vivo are tolerant. To study these processes, we have employed oligonucleotide microarray analysis to examine the progression of CD8 T cell differentiation upon stimulation with Ag and B7-1 in the presence or absence of either IL-12 or IFN α at 0, 24, 48, 72-h. We found that stimulation with Ag and B7-1 up- or down-regulated > than 2000 genes/ESTs at any of these time points. A total of 350 genes/ESTs were regulated in common by IL-12 and IFN α , including genes for effector function (granzyme B, FasL, IFN γ), survival, signal transduction, and transcription factors. Each cytokine also regulated unique sets of genes suggesting that the effectors generated may differ significantly depending upon the nature of the third signal. Clustering analysis revealed that the presence of the third signal acted to enhance and sustain expression of many genes (e.g. granzyme B, CD25) that are induced transiently at low levels by Ag/B7 stimulation, and also acted to induce expression of new genes (e.g. FasL, IFN γ). Furthermore, amongst the genes regulated in common by the two third signal cytokines, some were regulated in STAT4-dependent manner and others by STAT4-independent pathways. These results demonstrate that CD8 T cell differentiation initiated by the signal 3 cytokines involves programmed regulation of numerous genes needed for clonal expansion and development of effector functions, and suggest that some of the commonly regulated genes are also involved in determining whether Ag encounter ultimately leads to tolerance or memory.