

IDENTIFICATION OF DISTAL REGULATORY ELEMENTS THAT GOVERN ANTIGEN-SPECIFIC IFN- γ PRODUCTION IN VIVO.

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IFN- γ is the signature cytokine of CD8 and T helper 1 (Th1) CD4 cells, which are important in host defense against viruses and intracellular bacteria. However, with the exception of the promoter, little is known regarding regulatory elements that govern expression of the *Ifng* gene, and the limited information that is available is derived almost solely from studies in cell lines and primary cells cultured in vitro under polarizing conditions. We hypothesize that chromatin modifications, including dimethylation of lysine 4 on histone H3 (H3/K4^{me2}) and increased chromatin accessibility, are markers that signify regulatory elements governing IFN- γ production by antigen-specific T cells in vivo. To test this hypothesis, we isolated naïve LCMV antigen-specific CD4 and CD8 T cells, and either analyzed them directly or after they differentiated into effector T cells following adoptive transfer into mice that were infected with LCMV (Armstrong strain) for 8 days.

As expected, effector CD4 and CD8 T cells produced large amounts of IFN- γ , while naïve CD4 and CD8 T cells produced little or no IFN- γ , in response to stimulation with LCMV peptide. The ability to produce IFN- γ correlated with discrete peaks of H3/K4^{me2} in both effector CD4 and CD8 T cells. These peaks aligned with seven conserved non-coding sequences (CNS) found within 60kb upstream and downstream of the promoter. Naïve cells however lacked H3/K4^{me2} throughout the *Ifng* locus with the exception of two upstream regions termed 5'CNS3 and 5'CNS2. Each of these seven CNS regions were cloned upstream of the *Ifng* gene and transfected into EL-4 cells to assay for enhancer activity. 5'CNS1 and 3'CNS2 enhanced IFN- γ in the presence or absence of T-bet; 5'CNS2 enhanced IFN- γ only in the presence of T-bet. Together these data suggest that multiple distal regulatory elements are required for proper IFN- γ production during infection in vivo.