

Effects of GATA-3 downregulation in early T cell development

Deirdre D. Scripture-Adams and Ellen V. Rothenberg
Dept. of Biology, California Institute of Technology, Pasadena, CA 90025

T cells require an evolving pool of transcriptional regulators as their development proceeds, and as they pass through each maturational stage. GATA-3 is a dual Zn finger transcription factor, and is expressed throughout T cell development.

To identify the specific stages during which GATA-3 is essential for early T cell development, we are examining the gene regulatory and developmental effects of loss of GATA-3 in adh-2C2 (a DN3-like cell line), in fetal liver derived precursors, and in fetal thymocytes developing in OP-9 stromal culture systems. We are using retrovirally delivered GATA-3 specific siRNA to prevent GATA-3 protein synthesis during early T cell development. We are also using nucleofection technology to directly introduce siRNAs specific for GATA-3 into the nuclei of fetal liver derived precursors. Gene expression changes are monitored by quantitative PCR following reverse transcription of cellular RNA. Loss of GATA3 is detrimental in the earliest stages of T cell development. If GATA-3 is knocked down in fetal liver precursors, these cells fail to progress to the later stages of T cell development with normal efficiency and fail to proliferate normally. The requirement for GATA-3 for precursor progression is not relieved by forcing viability, as fetal liver precursors derived from bcl-2 transgenic mice in which GATA-3 has been knocked down retain the observed developmental block.

When early DN stage thymocytes are infected with a GATA-3 siRNA expressing virus, sorted into DN1, DN2, or DN3 populations, and cultured in the OP-9 stromal system, the cells fail to progress to the next stage of development as efficiently, relative to control infected cells. However, although the DN1 and DN3 show a dramatically reduced proliferation when GATA-3 is reduced, as well as reduced progression to subsequent developmental stages, the DN2 stage proliferates normally, even though these cells, too, show a slowed developmental progression.

We hope to understand the gene regulatory role of GATA-3 and its contribution to the specific gene regulation changes occurring during T lineage commitment and T specification by characterizing gene expression changes within these differentially affected developmental stages.