

Hardwiring T cell Function Through DNA Methylation

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During their development, lymphocytes must make a series of sequential, often binary, and generally irrevocable cell fate choices. First the T cell vs. B or NK cell lineages, then for T cells TCR $\alpha\beta$ or TCR $\gamma\delta$, CD4 or CD8, and Th1 or Th2 lineages. These choices reflect changes in overall programs of gene expression – some genes must be silenced and others must be turned-on. Once initiated, these new programs must be faithfully propagated from the parental cell to its progeny. Yet, sufficient plasticity must be maintained to allow subsequent cell fate choices to occur. With the exception of the choice between the TCR $\alpha\beta$ and TCR $\gamma\delta$ lineages, this does not reflect changes in the primary DNA sequence. Rather, choice is imposed epigenetically in a cell lineage-specific manner, but how such epigenetic changes are initiated and then faithfully propagated is uncertain. In the nucleus, DNA is intimately associated with histone proteins, which together constitute chromatin. Modifications in chromatin structure, and in particular post-translational modification of histones, and methylation of DNA on cytosines in CpG dinucleotide pairs, are proposed to provide an epigenetic code that helps to determine which genes are or can be expressed in particular cells. DNA methylation, particularly in promoter regions, represses gene expression and can be propagated in a heritable manner by the DNA methyltransferase Dnmt1, which copies the pattern of CpG methylation from the parental DNA strand to the daughter strand during S phase. We have focused our attention on the role of DNA methylation, because it is an epigenetic process for which an inheritance mechanism has been clearly defined.

Through conditional ablation of Dnmt1, we show that Dnmt1 and DNA methylation play a selective and developmental stage-specific role in the regulation of T cell lineage-specific gene expression. Inactivation of Dnmt1 in early DN thymocytes led to demethylation of CpG in the CD8 loci, followed by aberrant expression of CD8 on the majority of TCR $\alpha\beta$ cells. By contrast, this did not result in expression of CD8 on CD4⁺ TCR $\alpha\beta$ cells. DNA methylation also plays an important role in regulating CD4 vs. CD8 effector function. CD8 T cells ordinarily default to an effector program characterized by cytotoxicity and expression of IFN- γ . By contrast, naïve CD4 T cells normally produce low levels of the Th1 cytokine IFN- γ and the Th2 cytokines IL-4, IL-13 and IL-5, and only commit later to polarized production of one or the other. We find that the bias against IL-4 production by cells of the CD8 lineage normally commences at the SP thymocyte stage, and correlates with lower levels of GATA-3 expression and greater DNA methylation in the IL-4/IL-13 locus. Conditional ablation of Dnmt1 at the DP stage erases this bias, resulting in a dramatic upregulation of Th2 cytokine expression in Dnmt1-deficient CD8⁺ T cells and altering the chromatin structure within the IL-4/IL-13 locus. By contrast, GATA-3 and T-bet expression, and preferential IFN- γ , perforin or granzyme B expression by CD8⁺ T cells, is not affected. Our data are consistent with the notion that DNA methylation does not initiate but plays an essential role in maintaining the fidelity of T cell fate choices.

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