ABSTRACT

DETERMINING THE ROLE OF IRF6 IN T CELL DEVELOPMENT AND FUNCTIONAL COMMITMENT

By

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Interferon regulatory factor (IRF) is a protein family with nine members in mammals known to orchestrate the homeostatic mechanisms of host defense. There are functional and/or developmental defects of immune cells in the knockouts of eight family members. Like other family members, IRF6 is involved in regulating the cell cycle but in keratinocytes and mammary epithelial cell with mutations associated with squamous cell carcinomas. However, Irf6 is the only IRF known to be involved in morphogenesis. In humans, rare variants in IRF6 cause autosomal dominant orofacial clefting disorders while common variants contribute risk to non-syndromic forms. IRF6 is the only IRF family member with an as yet undetermined role in immunity.

Here, we used publically available microarray data to uncover a dynamic expression pattern for Irf6 during hematopoietic development. We found that Irf6 is expressed early in hematopoiesis in long term hematopoietic stem cells. Also we identified Irf6 expression in T cell lineage, including developing and functionally committed stages. Irf1, 2, 4, 8 are indispensable for a normal T cell development and differentiation. Genetic variants in IRF5, IRF7 and IRF8 are associated to autoimmune disorders of T cells. Furthermore, protein complexes between IRF6/IRF5 and IRF6/IRF8 were described. These data together with DNA conservation among the IRF members and structural homology with IRF5 strongly suggests a role for Irf6 in the immune system, specifically in T-cell development and functional commitment.

We utilized a mouse model to show that Irf6 was required for the regulation of thymocyte development. We found that Irf6 was expressed in the subcapsular region and medulla of the thymus. We further found that Irf6 regulated the distribution and proliferation of developing thymocytes. In addition, loss of Irf6 led to an increase in

double negative cells with a concomitant increase in TCRγδ. Loss of Irf6 also led to a reduction in double positive cells with no corresponding reduction in single positive cell maturation. Also, we found that Irf6 dose is critical in development of both CD4+ and CD8+ cells in an age-dependent manner. These data suggest a novel gene function for Irf6 in thymocyte development and indicate further studies of IRF6 variants that might increase the risk of autoimmune disease.

In the mouse, loss of Irf6 leads to perinatal lethality which hinders the ability to test the necessity of Irf6 in the functionally committed T helper (Th) subsets. In silico analysis suggested a model for Irf6 role in Th17/Treg balance. To test our hypothesis in vivo and overcome perinatal lethality, we employed an adaptive transfer of Irf6 knockout cells into lethally irradiated mice. Mice receiving Irf6 knockout cells had no deficit in restoration of lymphocyte production. In addition, we used two in vitro models to assess the necessity of Irf6 in the commitment of T helper cells. Using a stromal-free culture we found that naive T cells lacking Irf6 could be differentiated efficiently into Th1, Th2, Th17 and Treg using a specific cytokine cocktail. In vitro differentiation of dendritic cells showed significant increase of MHC-II expression after three days of culture. Irf6 might be involved in post-translational regulation of MHC-II. These data indicate that intrinsic Irf6 expression is not essential for T helper subset differentiation. However, a non-cell autonomous role for Irf6 in T cell differentiation through dendritic cells remains plausible.