Interestingly, comparison of the genomes of the two major serotypes of VSV, called *Indiana* (used by Otsuka et al. [2007]) and *New Jersey*, reveals that the miR-24 and miR-93 target sites are not conserved in the *New Jersey* serotype. This is especially striking for miR-24, which targets a highly conserved region of the genome, encoding the viral polymerase. The *New Jersey* strain contains a mutation at the same position as that selected by Otsuka et al. (2007) to inactivate miR-24 targeting, resulting in disruption of the seed binding of the miRNA, without affecting the coding sequence of the L gene. This illustrates how hazardous it would be for the host to rely on miRNAs to target viruses and questions whether the interaction of miR-24 and miR-93 with the genome of the *Indiana* serotype of VSV represents an adaptation of the virus to its host, rather than a defense mechanism. VSV may use miRNAs to limit the quantity of viral RNAs in infected cells, and control the extent of the inflammatory response, to protect its host. Indeed, field isolates of the *New Jersey* serotype have been shown to induce on average a ten-fold greater interferon response than isolates of the *Indiana* serotype (Marcus et al., 1998).

In conclusion, further studies are required to fully understand the role of Dicer and miRNAs in the intricate relationships between viruses and their mammalian hosts. No doubt that the Dicer-deficient mice described by Otsuka et al. (2007) will be a valuable asset to achieve this goal.

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Notch: Filling a Hole in T Helper 2 Cell Differentiation

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In this issue of *Immunity*, Amsen et al. (2007) and Fang et al. (2007) propose a direct role for Notch signaling in the expression of GATA-3 transcription factor and T helper 2 cell differentiation.

Notch signaling controls cell-differentiation processes in a wide variety of tissues throughout the life of multicellular organisms, including the lineage choice between T and B lymphocytes made by hematopoietic progenitors as they become more differentiated. Notch is a heterodimeric surface receptor consisting of an extracellular ligand-binding region noncovalently associated with a transmembrane polypeptide with a long intracellular tail. Mammals have four different Notch family members, Notch 1, 2, 3, and 4, which bind two conserved families of ligands, Jagged and Delta-like, encoded by two and three separate genes, respectively. Notch signaling is initiated by interaction of the extracellular region with its ligands, which are expressed on the surface of neighboring cells (Figure 1). The cleavage by γ-secretase releases the intracellular domain (ICD) of Notch from the membrane, allowing it to translocate into the nucleus. There, the ICD forms a complex with the ubiquitously expressed DNA-binding protein, recombination signal-binding protein-J (RBP-J), which is the mammalian ortholog of Su(H) (also known as CBF1 or CSL). Mastermind-like (MAML) binds to a groove at the interface between the ICD and RBP-J and, in turn, recruits critical coactivators, such as p300, that are required for transactivation of target genes.

The best-established role for Notch signaling in the hematopoietic system

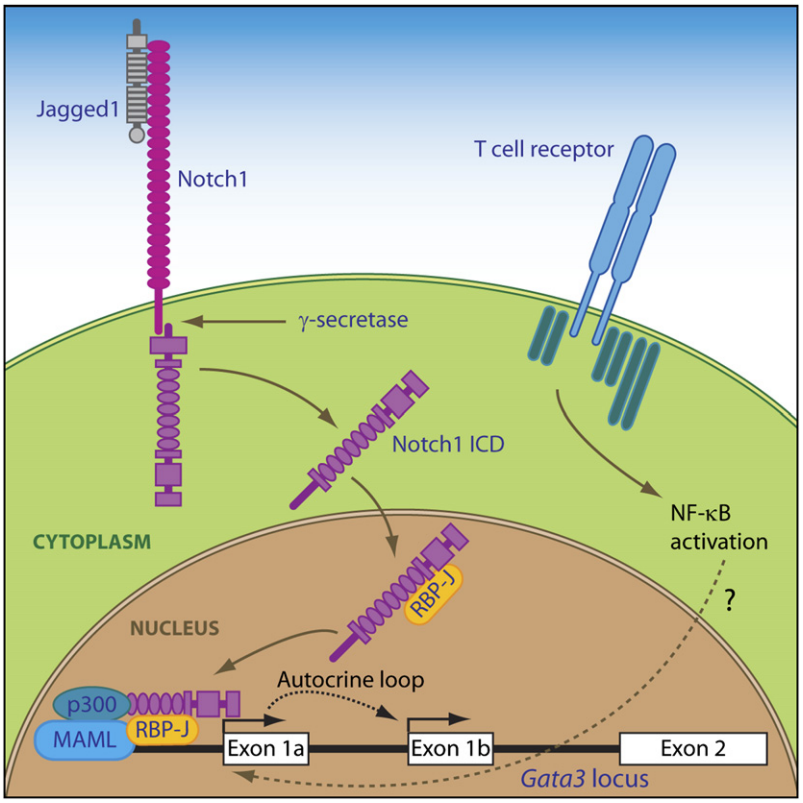

Figure 1. Notch Regulation in Th1 and Th2 Differentiation
Interaction of Notch1 with its ligands, Jagged1, results in the cleavage of the intracytoplasmic domain by γ-secretase. The resultant catalytic form of Notch (ICD) translocates into the nucleus. The ICD forms a complex with RBP-J, and the ICD-RBP-J complex preferentially binds to promoter of GATA-3 exon 1a and directly induces GATA-3 expression. Coordination with the T cell receptor signal via the activation of NF-κB pathway is essential for the induction of GATA-3 expression. However, the role of NF-κB in the formation of activation complex for the GATA-3 promoter remains unclear.

is the critical function of Notch1 in T cell-fate determination. Conditional loss-of-function analyses have shown that the Notch1-RBP-J signaling pathway is essential for the generation and differentiation of early T lineage progenitors in the thymus and that activation of this pathway simultaneously blocks B cell development. A major role of Notch-RBP-J signaling in early T cell development is the regulation of cell survival and expansion of pre-T cells at the T cell receptor β-selection checkpoint.

Outside of the thymus, Notch also contributes to many aspects of helper T cell differentiation. Gain-of-function studies with a soluble Delta 1-Fc fusion protein indicate that Notch has the capacity to drive T helper 1 (Th1) development (Maekawa et al., 2003). In vivo or in vitro treatment with an inhibitor of γ-secretase, whose cleavage activity is essential for formation of the ICD, leads to selective inhibition of Th1 responses through the blockade of T-bet expression (Minter et al., 2005). There are, however, data that question this model. Inhibition of Notch signaling by either the conditional deletion of RBP-J or expression of a dominant-negative form of MAML identified defects only in interleukin-4 (IL-4) production and Th2 responses (Amsen et al., 2004; Tanigaki et al., 2004; Tu et al., 2005). However, this impairment in RBP-J-deficient T cells is completely overcome by the addition of IL-4 during the induction phase. Based on this and other evidence, a recent report suggests that the Notch signal does not directly control Th2 differentiation, but rather regulates alternative mechanisms of IL-4 expression, with the initial source of IL-4 being restricted T cell subsets, such as memory type $CD4^+$ T cells or NKT cells (Tanaka et al., 2006). Therefore, a physiological role for Notch-RBP-J signaling in the regulation of Th2 differentiation still remains controversial; moreover, the molecular mechanism of Notch-mediated binding of RBP-J to target genes remains unresolved.

Amsen et al. (2004) have reported that Notch-RBP-J-mediated Th2 differentiation is regulated by antigen-presenting cell (APC)-derived instructive signals. According to their model, the instructive signal comes from Jagged1 expressed on dendritic cells (DCs). The interaction of Jagged1 with Notch during the initial stages of T cell activation controls the differentiation of naive $CD4^+$ T cells into Th2 cells by a mechanism independent of IL-4 and STAT6 signaling. These authors proposed that Notch-mediated binding of RBP-J to the distal 3′ *Il4* enhancer directly regulates IL-4 production at the transcriptional level. Notch induces IL-4 expression at least in part through response elements in the distal 3′ *Il4* enhancer within DNase I hypersensitivity site (HS)-V, which correspond to a well-conserved noncoding sequence (CNS) among mammals called CNS-2. The CNS-2 enhancer includes multiple conserved RBP-J-binding sites that are specifically responsive to Notch signals (Amsen et al., 2004; Tanaka et al., 2006). Therefore, the CNS-2 enhancer would be a target element for Notch-RBP-J-mediated Th2 differentiation induced by Jagged1-expressing DCs.

Amsen et al. (2007) and Fang et al. (2007) have now furthered our understanding of the IL-4- and STAT6-independent Notch-RBP-J-mediated Th2 differentiation mechanism. Both groups independently find that Notch-mediated binding of RBP-J to the regulatory region of exon 1a in the *Gata3* locus regulates GATA-3 expression in the absence of IL-4. GATA-3 is known to be a master regulator controlling Th2 differentiation. In this regard, both reports provide a new appreciation of the important role of Notch signaling in generating Th2 immunity. These authors found conserved RBP-J-binding sites in regions

of high DNA homology ~3, 5, and 10 kb upstream of the *Gata3* gene translational start site, and that Notch1 ICD and RBP-J were associated with the 10 kb exon 1a site. *Gata3* transcripts have two splice variants, 1a and 1b, and 1a is found in developing Th2 type cells whereas 1b is found in naive T cells. Dominant-negative MAML-treated $CD4^+$ T cells display a relative reduction of both the 1a and 1b transcripts. Expression of Notch ICD-induced exon 1a transcripts but has no effect on exon 1b expression. Similar exon 1a transcript induction is observed in STAT6-deficient $CD4^+$ T cells, indicating that Notch1 induces direct upregulation of the developmentally regulated *Gata3* exon 1a transcript in the absence of IL-4 and STAT6 signaling.

GATA-3 is a key regulator of the commitment process as naive T cells differentiate into Th2 cells because it can directly drive epigenetic modification of the IL-4 locus. However, except for the major pathway controlled by IL-4 receptor signaling in a STAT6-dependent manner, remarkably little is known about the molecular mechanisms regulating the expression of GATA-3 in T cells. Two modes of GATA-3 regulation have been proposed: an IL-4-dependent conventional mode operating in naive T cells and an IL-4-independent GATA-3 autocrine mode in developing Th2 cells (Asnagli et al., 2002). The IL-4-dependent mode controls the exon 1a transcript, whereas the GATA-3 autocrine mode controls the exon 1b transcript. Notch-mediated binding of RBP-J to the exon 1a promoter may function as a substitute for the IL-4-dependent mode in naive T cells. However, low amounts of Notch ICD induces GATA-3 expression in STAT6-deficient T cells, but the requirement for additional IL-4 in inducing maximum GATA-3 expression confounds the importance of the Notch signaling under physiological conditions. In the unpolarized state, $CD4^+$ T cells from the IL-4 and STAT6 loss-of-function mice show complete attenuation of GATA-3 induction. Therefore, although Notch signaling may be important in Th2 development, these data suggest that the amount of expression may be insufficient to drive maximum GATA-3 and Th2 responses in the absence of exogenous IL-4.

Contradictory data are found in some previous studies on the significance of IL-4-STAT6 signaling. Perhaps the most striking data show that in the STAT6-deficient mice, a small number of $CD4^+$ T cells express GATA-3 and secrete readily detectable amounts of IL-4 (Ouyang et al., 2000). Moreover, in vivo Th2 differentiation after infection with the parasitic nematode *Nippostrongylus brasiliensis* reveal clear redundancy of the IL-4 and STAT6 signaling pathway in $Il4^{-/-}$ and $Stat6^{-/-}$ mice. In this case, neither autocrine IL-4 from $CD4^+$ T cells nor alternative cellular sources of IL-4 seemed to be required for Th2 responses. Thus, Notch-mediated GATA-3 induction in $CD4^+$ T cells may fill the hole in IL-4-independent Th2 development.

The recognition of microorganisms by DCs during an innate response determines helper T cell differentiation. The particular class of pathogens encountered activates a different set of helper T cells. DCs recognizing DNA, RNA, or lipopolysaccharide (LPS) promote a Th1 response, whereas parasitic nematode or fungal infections enable DCs to induce strong Th2 responses. A well-characterized DC-derived instructive signal is IL-12, which is a potent inducer for Th1 differentiation. Notch ligands could be one of the DC-derived instructive signals that control T cell fate during helper T cell differentiation. There is evidence that LPS-induced Jagged1 expression promotes Th2 differentiation and that Delta4 expression promotes IL-12 production by $CD8^-$ DCs and subsequently controls Th1 differentiation. Extracts from *Schistome mansoni* eggs (SEA) downregulate the expression of Delta4, and thus SEA-treated DCs have been used as a Th2 adjuvant by Amsen et al. (2007). However, Fang et al. seem to show that GATA-3 induction could occur without involvement of Notch-ligand interactions, because plate-bound TCR crosslinking induces marked GATA-3 exon 1a expression in wild-type T cells (Fang et al., 2007). Thus, the mechanism by which Notch is activated in purified $CD4^+$ T cells is still unclear.

In considering the role of Notch signal in helper T cell differentiation, an important question is whether Notch signal is required for Th1 or Th2 cell differentiation or both pathways. Both Amsen et al. (2007) and Fang et al. (2007) clearly demonstrate that loss of Notch signaling impaired Th2 cell differentiation, but the studies did not address Th1 cell differentiation because the experimental strategy was designed for Th2 polarization. Therefore, it still remains possible that Notch has some role in Th1 responses although Amsen et al. (2007) show that Notch1 and Notch2 deficiency does not impair IFN-γ production. Thus, although the role of Notch signaling in Th2 differentiation is the regulation of initial IL-4 source from memory T cells and the direct regulation of GATA-3, the role of Notch in Th1 differentiation still remains an open question.

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