

Review



Cite this article: Brandstadter JD, Maillard I. 2019 Notch signalling in T cell homeostasis and differentiation. *Open Biol.* **9**: 190187. <http://dx.doi.org/10.1098/rsob.190187>

Received: 9 August 2019

Accepted: 8 October 2019

Subject Area:

immunology

Keywords:

Notch, T cell, infection, autoimmunity, alloimmunity, transplantation

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Notch signalling in T cell homeostasis and differentiation

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The evolutionarily conserved Notch signalling pathway regulates the differentiation and function of mature T lymphocytes with major context-dependent consequences in host defence, autoimmunity and alloimmunity. The emerging effects of Notch signalling in T cell responses build upon a more established role for Notch in T cell development. Here, we provide a critical review of this burgeoning literature to make sense of what has been learned so far and highlight the experimental strategies that have been most useful in gleanng physiologically relevant information. We outline the functional consequences of Notch signalling in mature T cells in addition to key specific Notch ligand–receptor interactions and downstream molecular signalling pathways. Our goal is to help clarify future directions for this expanding body of work and the best approaches to answer important open questions.

1. Introduction

Notch, an evolutionarily conserved cell-to-cell signalling pathway, plays multiple functions at selected stages of innate and adaptive immune cell development, as well as in regulating mature immune cell function. Through its involvement in both developing and mature immune cells, Notch is emerging as a critical actor in host defence and immune pathology.

Notch was first discovered to influence haematopoiesis based on its oncogenic role in T cell leukaemia [1,2], a corruption of its role in T cell development [3,4]. However, an emerging role of Notch signalling in mature T cells during homeostasis and immune responses in peripheral tissues has now come into view. Work has begun to illuminate how cell-type and context-specific Notch signalling shapes T cell immune responses while also driving T cell-mediated pathologies.

Here, we will review the literature to summarize prior work and to weigh available evidence for how Notch influences mature T cells in the periphery. In particular, we will discuss the importance of *in vivo*, loss-of-function strategies that have proven most reliable as opposed to *in vitro* and gain-of-function experiments. Overall, we aim to provide a clear picture of what has been established in the field and identify larger themes for how Notch functions in mature T cells.

2. Overview of Notch signalling

Notch is a highly conserved cell–cell communication pathway driven by juxtacrine Notch ligand–receptor interactions (figure 1). The four mammalian heterodimeric Notch receptor paralogs (Notch1–4) interact with one of five Notch ligands in the Jagged (Jag1 and Jag2) and Delta-like (Dll1, Dll3 and Dll4) families [5,6]. Notch ligands activate Notch signalling, except Dll3 which is thought to act as a natural antagonist of the pathway [5]. A mechanical force induced by ligand–receptor interactions triggers sequential proteolytic cleavages in the Notch receptor. First, an ADAM-family metalloprotease (ADAM10) targets

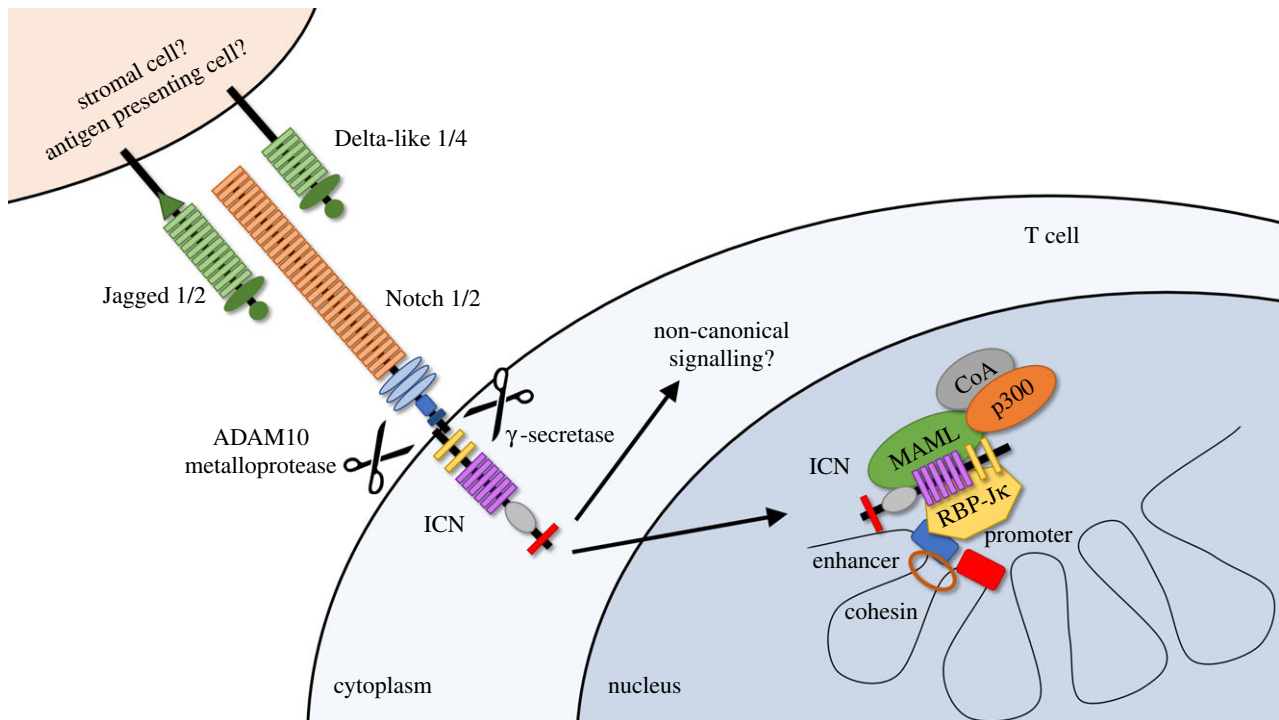


Figure 1. Overview of Notch signalling. Mammalian Notch receptors expressed by mature T cells receive juxtacrine signals from four activating ligands (Jagged 1/2 or Delta-like 1/4) expressed on adjacent cells (either stromal cells in secondary lymphoid organs or professional antigen-presenting cells). Ligand/receptor binding triggers sequential proteolytic cleavage of the Notch receptor, first by the ADAM10 metalloprotease and then by the γ -secretase complex. These cleavages release intracellular Notch (ICN) into the cytoplasm where it enters the nucleus to form a transcriptional activation complex with the DNA-binding transcription factor RBP-J κ and a member of the Mastermind-like (MAML) family, which in turn recruit additional transcriptional coactivators (CoA). The Notch transcriptional complex modifies chromatin structure to form clusters of enhancers and promoters and affect transcription. In some instances, ICN was reported to signal through non-canonical RBP-J κ /MAML-independent pathways.

the receptor's membrane-proximal extracellular domain, rendering it susceptible to the γ -secretase complex, which induces intramembrane proteolysis and releases intracellular Notch (ICN) into the cytoplasm. After migration into the nucleus, ICN interacts with the DNA-binding transcription factor RBP-J κ and recruits a transcriptional co-activator of the Mastermind-like family (MAML1-3) [5–9]. MAML in turn interacts with other transcriptional activators, including chromatin-modifying enzymes such as histone acetyltransferases and other components of the transcriptional activation machinery.

Although transcriptional regulation by Notch signalling has been studied in multiple contexts, data from studies in Notch-driven cancers (e.g. T cell acute lymphoblastic leukaemia, B cell lymphoproliferative disorders, breast cancer) have provided the most detailed information to date. In T cell leukaemia, ICN/RBP-J κ complexes bind thousands of sites in the genome, although less than 10% are actually dynamically regulated upon blockade of Notch signalling. Many of these dynamically regulated sites cluster with distant enhancers where Notch occupancy is associated with alterations in chromatin regulation [10]. Interestingly, recent work illuminated how oncogenic Notch can influence chromatin looping to reposition enhancers into '3D cliques' of interacting enhancer/promoter spatial clusters (figure 1) [11]. This pattern of activity broadens the mechanisms of Notch-mediated control of gene expression beyond its effects on a static cohort of target genes, suggesting that context from other signals might be important to determine patterns of enhancer activation and chromatin repositioning. Thus, individual Notch target genes are predicted to be highly context-dependent.

Notch signalling is regulated by strict temporal and spatial control of Notch ligand expression by selected cells. For example, high levels of Dll4 ligands are expressed in thymic epithelial cells, creating an anatomical niche for Notch signalling in T cell development [12–14]. Notch signals are also regulated by O-glycosylation of serine or threonine residues in the epidermal growth factor (EGF) domains of the receptor. Loss of O-glycosylation phenocopies loss of Notch signalling [15]. O-glycosylation can be elongated by the addition of N-acetylglucosamine by the glycosyltransferase Fringe, which biases Notch receptors to preferentially signal via Delta-like over Jagged ligands [16]. Genetic deletion of *Fringe* genes typically induces Notch loss-of-function phenotypes, including effects on T cell development [17].

After initial proteolytic activation, Notch signalling is regulated by the rapid targeting of active ICN for proteasomal degradation through its C-terminal PEST domain via the FBW7 E3 ubiquitin ligase. *FBW7* mutations and truncations of the *NOTCH1* PEST domain have been identified in Notch-driven T cell acute lymphoblastic leukaemia (T-ALL). Over 50% of all T-ALL patient samples and cell lines carry activating *NOTCH1* mutations, including PEST truncations and membrane-proximal mutations that induce receptor activation [2,18,19].

Notch signalling is essential for T cell development, and its effects can be corrupted to drive T-ALL [2]. In this context, which is not the central focus of our review, key mutational mechanisms of oncogenic Notch activation include point mutations affecting the *NOTCH1* extracellular heterodimerization domain, thus leading to constitutive proteolytic receptor activation; and mutations truncating the intracellular

C-terminal NOTCH1 PEST domain, increasing its half-life after activation. These two classes of mutation can occur together in the same *NOTCH1* allele, suggesting cooperativity. Altogether, activating *NOTCH1* mutations and related genetic events have been reported in at least 60–70% of primary T-ALL cases, consistent with a dominant oncogenic function of Notch signalling in this disease.

Similarly, *NOTCH1* and *NOTCH2* mutations are found in different B cell lymphomas, with *NOTCH1* being recurrently mutated in chronic lymphocytic leukaemia (CLL) and *NOTCH2* in marginal zone lymphoma [20–25]. These mutations are typically frameshift or nonsense mutations decreasing ICN turnover by truncating the C-terminal PEST domain to make ICN less vulnerable to degradation. Interestingly, even independent of explicit *NOTCH1* mutations, roughly 50% of CLL cases in one series had a *NOTCH^{high}* gene expression signature and another series found increased activated NOTCH1 by immunohistochemical staining in over 80% of cases, suggesting that Notch ligands drive signalling in CLL even with a non-mutated Notch receptor [26,27].

3. T cell development

T cell development proceeds after lymphocyte progenitors differentiate from bone marrow haematopoietic stem cells and migrate to the thymus [28]. Specialized thymic epithelial cells induce T cells to develop along an organized stepwise approach. The process begins with early thymocytes that are double-negative for cell surface expression of CD4 and CD8. The cumulative effect of this process is the generation of CD4⁺ and CD8⁺ T cells with a diverse repertoire capable of recognizing peptide–MHC antigen complexes.

Notch has been traditionally described for its role in early T cell development. This topic has been reviewed elsewhere [29], but we will highlight critical observations relevant for the interpretation of insights in mature T cells. Notch signalling plays a critical role during T lineage commitment in the thymus, a role that was first thought to be at the expense of B lymphopoiesis. Genetic inactivation of *Notch1* or its downstream transcriptional machinery results in a hypoplastic thymus permissive for intrathymic B lineage development [3,30]. Reciprocally, overexpression of constitutively active Notch results in the development of thymic-independent T cells and suppression of bone marrow B cell development. However, Notch also exerts negative regulation of myeloid fates in the thymus [31,32]. Thus, the original model of Notch controlling a T/B binary cell fate decision gave rise to a more complex pattern of Notch driving T lineage development while repressing multiple alternative cell fates. Interestingly, recent work reported a role for Notch signalling during initial prethymic steps of lymphoid and T lineage specification in the bone marrow, suggesting that endothelial and non-endothelial mesenchymal elements in the bone marrow microenvironment can provide signals to lymphoid progenitors through the Dll4 Notch ligand [33,34]. Yet, the abundance of Dll4 expression in the bone marrow and/or its precise microanatomical distribution must remain tightly controlled, as broad Dll4 derepression in LRF-deficient mice can lead to full-fledged extrathymic T cell development at the expense of other lineages [35,36].

As lymphoid progenitors enter the thymus, they encounter dense expression of Notch ligands on cortical thymic

epithelium that is essential for thymopoiesis [3,13,14,37,38]. Notch signals persist until the pre-T-cell receptor checkpoint, after which Notch signalling intensity decreases [39–42]. Double-positive thymocytes receive little Notch signalling under physiologic conditions. Notch is dispensable for positive and negative selection [43–44] and for release of mature T cells into the periphery.

4. Notch signalling impacts mature T cell function

An important role for Notch signalling in mature T cells has now been established in models of host defence, autoimmunity and alloimmunity (table 1). Work describing how Notch functionally impacts mature T cells began with overexpression experiments, leading to conclusions that were then revised based on *in vivo*, loss-of-function strategies.

Early evidence for a role for Notch in mature T cells suggested a tolerogenic effect. Overexpression of Jagged1 in professional antigen-presenting cells (APCs) expressing a host dust mite antigen conferred tolerance and drove CD4⁺ T cell differentiation into immunosuppressive regulatory T cells (Treg) [72]. Similar tolerance and Treg differentiation were observed when Jagged1-overexpressing APCs presented Epstein–Barr virus antigens to autologous or allogeneic T cells in culture, suggesting a role for Notch in tolerance to virus and alloantigens [73,74]. However, these overexpression models did not correlate well with other *in vitro* findings that Notch ligands in APCs were upregulated in response to pathogen encounter, suggesting proinflammatory functions [75–77].

More recently, genetic and pharmacologic *in vivo* loss-of-function strategies have shown a dominant proinflammatory effect of Notch signalling in mature T cells. Notch signalling appears important for a robust T cell response in host defence (table 1). For example, conditional deletion of *Notch1* and *Notch2* (but not either in isolation) in mouse CD4⁺ T cells conferred susceptibility to *Leishmania* infection [47], suggesting that Notch is essential to generate IFN γ -secreting cells to control the parasitic infection. Interestingly, the *Dll1* gene has been linked to susceptibility to visceral leishmaniasis [78]. In other infection models, *Notch2* loss impaired CD8⁺ T cell function and conferred susceptibility to the parasite *Trypanosoma cruzi* [45]. Genetic *Notch1* and *Notch2* inactivation impaired CD8⁺ T cell response to both *Listeria* and influenza infection, ultimately impairing pathogen clearance [48,50]. *In vivo* administration of a neutralizing antibody against Dll1 also impaired clearance of influenza infection and resulted in higher mortality and lower production of IFN γ , a finding replicated upon *in vivo* pan-Notch inhibition via γ -secretase inhibitors (GSI) [49]. Expression of a dominant-negative form of MAML (DNMAML) in CD4⁺ T cells to block Notch signalling resulted in higher fungal burdens from *Cryptococcus neoformans* [51]. Similarly, a neutralizing antibody against Dll4 impaired immune control of mycobacteria-elicited pulmonary granulomatosis with lower levels of otherwise protective cytokines found in these mice [46]. Impairment in the CD4⁺ T cell response was also seen with model antigens upon conditional deletion of *Notch1/2* or *Rbpj* (versus deletion of *Dll4* in APCs) [70,71]. Altogether, loss-of-function approaches have established proinflammatory effects of Notch signalling on mature T cells in host defence, but with context-specific consequences.

Table 1. Experimental evidence supporting a role for Notch signalling in mature T cell function.

	disease model	<i>in vivo</i> , loss-of-function strategy	outcome	citation
host defence	<i>Trypanosoma cruzi</i> infection	deletion of <i>Notch2</i> in CD4 ⁺ T cells	impaired survival; impaired GZMB expression	[45]
	mycobacteria-elicited pulmonary granulomatosis	systemic anti-Dll4 treatment	larger granulomas; decreased Th17 cytokines (IL-17/17A/F, -6, -21)	[46]
	<i>Leishmania major</i> infection	deletion of <i>Notch1</i> and <i>Notch2</i> in T cells	susceptibility to infection, impaired IFN γ	[47]
	Influenza infection	deletion of <i>Notch1</i> and <i>Notch2</i> in CD8 ⁺ T cells; systemic anti-Dll1 treatment; GSI treatment	impaired viral clearance; decreased terminal effector cell differentiation, impaired IFN γ ; impaired survival	[48,49]
	<i>Listeria monocytogenes</i> infection	deletion of <i>Notch1</i> and <i>Notch2</i> in CD8 ⁺ T cells	decreased short-term effector cell differentiation, impaired IFN γ	[50]
	<i>Cryptococcus neoformans</i> infection	DNMAML expression in T cells	increased fungal burden, impaired Th1/Th2 response	[51]
autoimmunity	experimental autoimmune encephalomyelitis (multiple sclerosis)	GSI treatment; systemic anti-Dll4 treatment; systemic anti-Notch3 treatment; DNMAML expression in T cells	decreased disease scores, impaired IFN γ ; impaired IL-17A/IFN γ in CNS	[52–57]
alloimmunity	graft-versus-host disease	DNMAML expression in donor T cells; deletion of <i>Notch1</i> and <i>Notch2</i> or <i>Rbpj</i> in donor T cells; systemic anti-Notch1/2 or anti-Dll1/4 treatment; deletion of <i>Dll1/4</i> in recipient CCL19 ⁺ stromal cells	decreased GVHD severity scores, increased survival, impaired IFN γ , increased Treg expansion	[58–66]
	heart transplant	systemic anti-Dll1 treatment + CTLA4-Ig/CD28KO; systemic anti-Dll1/4 treatment; DNMAML expression in T cells; systemic anti-Notch1 \pm CTLA4-Ig treatment; Deletion of <i>Notch1</i> in Tregs	delayed cardiac allograft rejection, impaired GZMB; impaired IFN γ /IL-4, decreased graft infiltration, decreased donor-specific alloantibodies	[67–69]
	lung transplant	systemic anti-Notch1 + anti-CTLA4-Ig treatment	preserved airway patency with less lymphocytic infiltrations and delayed lung allograft rejection	[69]
	human skin graft to mouse with chimeric human haematopoietic system	systemic anti-Notch1	decreased T cell infiltration, greater proportion of Tregs infiltrating, improved vascularity	[69]
model antigens	H-Y antigen	deletion of <i>Dll4</i> in DCs	impaired CD4 ⁺ T cell activation and IL-2 production	[70]
	keyhole limpet Haemocyanin alum, <i>Schistosoma mansoni</i> egg extract	deletion of <i>Notch1</i> and <i>Notch2</i> or <i>Rbpj</i> in T cells	impaired CD4 ⁺ T cell activation	[71]

In vivo loss-of-function experiments in autoimmunity models also revealed a dominant proinflammatory role of Notch signalling in mature T cells (table 1). In a mouse model of multiple sclerosis, Notch inhibition by γ -secretase inhibitors (GSI) slowed disease progression [52]. This exciting finding was supported by later studies suggesting roles for Dll4 and Notch3 via systemic blocking antibodies [52–56]. We also observed

dramatic disease protection in mice with T cells deprived of Notch signalling via expression of DNMAML [57]. This protection appeared independent of T cell activation and differentiation in secondary lymphoid organs, although Notch-deprived, myelin-reactive T cells in the central nervous system did not produce inflammatory IL-17A and IFN γ , suggesting local effects of the Notch pathway in the target

organ. Clinical correlations and other associative studies also suggest roles for Notch in other autoimmune disorders, including rheumatoid arthritis and systemic sclerosis, and indeed GSI treatment allowed for disease improvements in mouse models [79–81]. However, these autoimmune disease models require further work using more targeted loss-of-function strategies to clarify the role of Notch signalling in this context.

Notch signalling in T cells is a critical regulator of alloimmunity during both solid organ transplant rejection and graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation, which we have reviewed elsewhere [82]. As in models of host defence, early research relied on overexpression of Notch ligands, which suggested a tolerogenic effect of Notch signalling in T cells. Fibroblasts overexpressing both Dll1 and MHC-loaded alloantigens could be adoptively transferred to mice with cardiac allografts to delay CD8⁺-mediated graft rejection [83]. Furthermore, Jagged1-overexpressing APCs (alongside CD40 blockade) delayed cardiac allograft rejection [84]. However, as observed in infection models and autoimmunity, *in vivo* loss-of-function approaches instead revealed a dominant proinflammatory role of Notch signalling in alloimmunity (table 1). The use of Dll1-blocking antibodies alongside costimulation blockade delayed rejection and reduced inflammatory cytokine secretion in a MHC-mismatched heart transplantation mouse model [67]. Similarly, more complete Notch blockade with either Dll1 and Dll4 blocking antibodies or T cell-specific ablation of Notch signalling using DN MAML delayed cardiac allograft rejection without costimulation blockade [68]. Interestingly, short-term Dll1/4 blockade during the peritransplant period was sufficient to confer protection against CD4⁺ T cell-mediated rejection in this model. Similarly, recent work showed a role for peritransplant Notch1 antibody blockade in delaying graft rejection in models of MHC-mismatched heart and lung transplantation in addition to a model where human skin is grafted onto a mouse with a chimeric human haematopoietic system [69]. This work found that Treg depletion through anti-CD25 antibodies cancelled the beneficial effects of Notch blockade and that conditional *Notch1* deletion in Tregs could similarly delay graft rejection.

GVHD is the life-threatening consequence of an alloimmune response following bone marrow transplantation, whereby donor T cells attack recipient tissues [82]. We found dramatic protection against GVHD in MHC-mismatched transplants when Notch signalling was blocked in donor T cells via either conditional DN MAML expression or loss of RBP-J κ [58,59]. We phenocopied these effects via conditional *Notch1* and *Notch2* deletion in donor T cells [60,61]. We also obtained similar protection using blocking antibodies against Dll1 and Dll4 ligands. A dominant role was observed for Notch1 and Dll4. Interestingly, transient Dll1/4 blockade at the time of transplant conferred long-term protection from GVHD. Other groups identified protection from GVHD upon abrogation of Notch signalling in mature T cells in their models [62–64]. While one model used conditional loss of RBP-J κ in Tregs to suggest that Notch signalling in Tregs is the essential driver of GVHD protection [64], side-by-side conditional deletion of Notch signalling in Tregs alongside TconvS revealed that Notch inhibition in TconvS remained essential for conferring protection from GVHD [65].

Overall, across models of infection, autoimmunity and alloimmunity, *in vivo* loss-of-function experiments revealed a pleomorphic proinflammatory function of Notch signalling

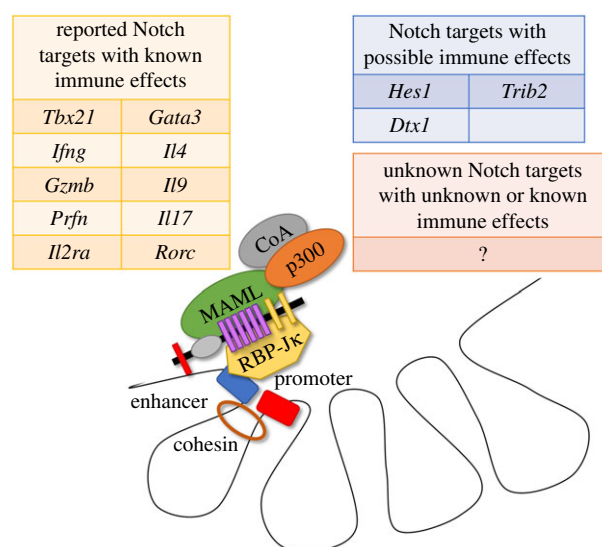


Figure 2. Classes of possible Notch transcriptional target genes in mature T cells. Notch dynamically regulates transcription by binding RBP-J κ and a member of the MAML family to recruit additional coactivators and globally alter chromatin structure at clusters of enhancers and promoters. Most genome-wide research into Notch transcriptional targets has been performed in developing thymocytes and Notch-driven T cell leukaemia, with unclear significance for mature T cells. In this context, Notch has been shown to alter transcription of selected immunologically important genes related to T cell differentiation and function. Additionally, a small number of classically described Notch targets have purported immunological effects in mature T cells (e.g. *Hes1*, *Dtx1*, *Trib2*). However, many immunologically relevant Notch transcriptional targets probably remain to be defined in mature T cells.

in mature T cells. Collectively, this work shows the power of *in vivo* loss-of-function models to uncover physiologic roles for Notch signalling as overexpression, gain-of-function experiments had suggested an otherwise contradictory tolerogenic role for Notch in these settings.

5. Notch intracellular signalling pathways in mature T cells

Mature naive CD4⁺ and CD8⁺ T cells express Notch1 and Notch2 receptors [77,85–87], with upregulated expression following T cell receptor (TCR) stimulation [88]. Increased cleaved Notch1 was also observed following antigenic stimulation of CD4⁺ T cells [85]. Besides Notch1 and Notch2, Notch3 has been explored for its effects in developing thymocytes [89] and in T-ALL [90], and at least one group has also detected it in cultured mature T cells [88]. However, while systemic Notch3 blocking antibodies was protective in a mouse model of multiple sclerosis [55], no genetic loss-of-function data have been reported to date demonstrating its role in mature T cells. In contrast, forced Notch3 overexpression experiments have suggested a potential role that needs to be further explored [91,92].

In mature T cells, as in developing thymocytes, Notch signals via proteolytic cleavage of the receptor to release ICN, which translocates to the nucleus and initiates transcriptional activation of target genes. The nature and regulation of Notch transcriptional targets remains poorly understood in mature T cells (figure 2). Gain-of-function experiments have shown increased *Hes1* and *Dtx1* expression upon Notch ligand

exposure [73,93], and we have observed alterations in *Dtx1*, *Hes1*, *Il2ra* and *Trib2* gene expression using an *in vivo* model of alloimmunity with Notch ligand blockade—all genes previously identified as Notch targets in other contexts [66]. Others found ICN capable of binding the *Gzmb* promoter [45]. More recently, using bulk RNA sequencing, we found that Notch inhibition during allogeneic bone marrow transplant impaired transcription of a subset of Myc-regulated genes and altered a transcriptional programme largely independent of targets identified in T cell leukaemia and thymopoiesis [65].

Notch has been shown to regulate a number of genes necessary for guiding different T cell differentiation fates, as discussed further below. Indeed, Notch has been shown to target *Tbx21*, *Il4*, *Gata3-1a*, *Il17a* and *Rorc* (figure 2) [52,77,94,95]. In addition, Notch has been shown to directly regulate *Ifng* via binding to the *Ifng* CNS-11 enhancer [96]. However, transcriptional targets of Notch signalling remain much better studied in developing thymocytes and malignant cells. It is unclear to what extent these findings can be applied to mature T cells as many targets have been found to be cell-type and context-dependent. For example, *IL7RA* has been a well-validated Notch target gene in thymocyte progenitors and T-ALL, although it remains to be explored in mature T cells [10,97–99]. Regardless, it seems likely that, as shown in T cell leukaemia, only a small fraction of ICN/RBP-J κ binding sites is dynamically regulated [10].

Emerging data also suggest a potential role for non-canonical Notch signalling whereby Notch exerts biologic functions independently of ICN translocation to the nucleus to drive transcription with RBP-J κ , MAML and other coactivators [52,93,100–102]. Fruit flies and cultured mammalian cells were first reported to respond to Notch signals independent of its transcriptional activation complex [103,104]. Roles for non-canonical Notch signalling pathways have also been proposed in mature T cells [52,93,101,102]. These pathways include signalling via *Tbx21* upregulation and NF- κ B activation. Indeed, ICN was found to complex on the *Tbx21* promoter and interact directly with NF- κ B pathway intermediates, supporting a role for non-canonical Notch signalling in mature T cells.

Overall, the most rigorous strategy to identify non-canonical Notch signalling is to show phenotypic discordance between genetic or pharmacologic abrogation of Notch receptors and deletion or impairment of its transcriptional machinery (either via loss of RBP-J κ or DN MAML expression). For example, a role for non-canonical Notch signalling was suggested in controlling IFN γ production by CD4⁺ T cells during *Leishmania* infection [47]. *Notch1* and *Notch2* inactivation in T cells conferred susceptibility to infection, but mice with a similar conditional loss of RBP-J κ remained protected. These data provide evidence for a non-canonical pathway of Notch signalling in this setting, although precise molecular mechanisms remain to be identified. A similarly rigorous genetic approach found a limited role for non-canonical Notch signalling in Tregs, although canonical signalling remained the dominant pathway [64]. After finding that *Notch1*-deficient and RBP-J κ -deficient Tregs had enhanced expansion and tolerance induction, the authors forced ICN expression in Tregs to drive Treg dysfunction and autoimmunity. Loss of RBP-J κ in ICN-expressing cells rescued many of these phenotypes (favouring a canonical pathway). However, selected findings were unaffected, such as impaired demethylation of

Foxp3, suggesting a role for non-canonical Notch signalling. Overall, although non-canonical signalling occurs in some instances in mature T cells, confirmatory *in vivo*, loss-of-function studies remain essential to determine the relevance of this phenomenon in most contexts.

6. Notch signalling in T cell differentiation

The use of loss-of-function, *in vivo* modelling also provided clarity for how Notch functions in mature T cell differentiation. Differentiation is a polarization of the immune response toward a particular set of functions most effective at combating a particular type of threat. During differentiation, these CD4⁺ T cells respond to APCs via juxtacrine cell–cell signals and soluble cytokines. Two of the most prominent differentiation states include the T helper 1 (Th1) cell fate where CD4⁺ T cells, driven by the transcription factor T-bet (encoded by *Tbx21*), make IFN γ to combat viruses and intracellular pathogens [105] and the Th2 cell fate where T cells combat helminth parasites via production of IL-4, IL-5 and IL-13 through the GATA3 transcription factor [106–108]. Although others have reviewed the role of Notch in T cell differentiation, we will focus on critical evaluation of the literature to weigh seemingly contradictory evidence and draw out larger emerging themes [109].

An early model for how Notch might be influencing mature T cell differentiation suggested that Notch was a bipotential switch, toggling between Th1 and Th2 cell fates, with consequences dependent on the nature of the Notch ligands involved. Indeed, early work found that forced overexpression of Notch ligands Dll4 and Jagged1 in APCs resulted in ligand-specific differentiation of mature CD4⁺ T cells to Th1 or Th2 cell fates, respectively [77].

However, a more recently proposed model whereby Notch acts as an unbiased amplifier of T cell differentiation may best correspond with available data [96]. The amplifier model helps synthesize otherwise conflicting findings that Notch controls gene expression of opposing transcriptional differentiation programmes, including playing a role in targeting *Tbx21*, *Il4*, *Gata3-1a*, *Il17a* and *Rorc* [52,77,94,95]. An amplifier model clarifies seemingly contradictory data that suggested roles for Notch signalling in promoting either Th1 or Th2 responses under different polarizing conditions. For example, Notch was reported to promote Th1 differentiation as Dll1 could drive Th1 commitment *ex vivo* [92], while Notch blockade via GSI suppressed Th1 differentiation *in vivo* [52]. Others proposed that Notch promoted Th2 differentiation, as DN MAML expression led to impaired IL-4 and Th2 cytokine production and impaired defence against *Trichuris muris* [94,110]. An amplifier model, whereby Notch helps sustain expression of different gene targets for both Th1 and Th2, synthesizes these data and makes sense of how a fundamental cell signalling pathway can drive differentiation of several distinct lineages. In other words, Notch regulates distinct transcriptional programmes for differentiation to various T cell fates by sensitizing T cells to cytokine-derived and other regulatory signals. This model was proposed after experiments using conditional expression of DN MAML to ablate Notch signalling found that Notch had no impact on Th2 initiation following infection with the helminth *T. muris*, but did affect maintenance of Th1 and Th2 programmes [96]. Notch was also shown to be needed for the maintenance of the Th17 response (another

differentiation state characterized by IL-17 expression that is important in immunity against extracellular pathogens and associated with autoimmune conditions). The amplifier model is further supported by mechanistic work showing that Dll4 expression on APCs could activate CD4⁺ T cells via augmenting PI3 K pathway signalling downstream of CD28 co-stimulation through a pattern of activity reminiscent of costimulatory signals [70].

Beyond Th1, Th2 and Th17, Notch has also been linked to other differentiation states. Th9 responses, so called for the production of the cytokine IL-9, are related to Th2 in anti-helminth immunity and appear to be implicated in certain autoimmune diseases. It also requires Notch signals as loss of Notch1/2 receptors abrogated the development of Th9 cells, while ICN, RBP-Jκ and Smad3 (downstream of TGFβ) were all found to cooperatively bind the *Il9* promoter [53].

Notch also regulates T cell differentiation into follicular T helper cells (Tfh). Tfh cells specialize in helping B cells during isotype class switching and affinity maturation via CD40 L and secreted cytokines [111]. *Notch1/2* deletion in T cells resulted in decreased numbers of Tfh and IL-21 production in response to parasitic infection and hapten immunization [112], translating into impaired germinal centre formation and IgG1 production independently of Notch's effects on IL-4.

Notch signalling may also regulate Treg differentiation, which mediates peripheral tolerance via FoxP3-dependent mechanisms [113]. Overexpression studies first suggested that Jagged ligands were capable of promoting Treg expansion [73]. Indeed, cultured T cells exposed to TGFβ, which assume a Treg-like phenotype, lost this ability upon addition of GSI [114]. However, *in vivo* loss-of-function approaches suggested an alternative role for Notch in Tregs. Genetic *Notch1* or *Rbpj* inactivation instead led to a 'super-regulatory' phenotype [64]. Consistent with this work, blocking Notch signalling *in vivo* through genetic or pharmacologic means during allogeneic bone marrow transplantation resulted in Treg expansion [58,66]. Overall, *in vivo* work suggests that Notch signalling curtails Treg function.

7. Source and specificity of Notch ligands

In vivo T cell conditional abrogation of Notch receptors and of the Notch transcriptional machinery (either via RBP-Jκ loss or DNAM1 expression) has been the most robust approach to glean insights into the pleiotropic effects of Notch in mature T cells. Ligand-targeted genetic strategies have proven initially more challenging, given limited insight into cellular sources of ligands and possibly different or redundant effects from different ligands. Loss-of-function strategies targeting ligands therefore mostly relied on systemic blocking antibodies. Otherwise, many experiments have depended upon ligand over-expression models, which are hypothesis-generating but less physiologically relevant.

In development, the thymic epithelial cell niche where Notch ligands are provided to early thymocytes has been well characterized (figure 3) [12–14]. The corollary niche for mature T cells in the periphery is only beginning to emerge. Initial focus centred on professional APCs as the suspected cellular source of Notch ligands, as they provide antigen and multiple costimulatory signals during T cell activation. Indeed, Notch ligands are expressed by APCs and *in vitro* experiments where Notch ligands were induced by Toll-like

receptor (TLR) agonism suggested that APCs could be the cellular source of ligands [77,115,116]. Furthermore, Notch ligands upregulated in a subpopulation of APCs from mice receiving allogeneic bone marrow transplantation could activate T cells *in vitro* in a ligand-dependent fashion [63].

However, the physiologic relevance of *in vitro* experiments suggesting APCs as the source of Notch ligands for mature T cells had not been rigorously confirmed with *in vivo* experimentation. On the contrary, loss-of-function *in vivo* experiments uncovered a non-haematopoietic source of Notch ligands. This was first suggested for marginal zone B (MZB) cells—another lymphocyte population—as bone marrow chimeras identified the source of Dll1 necessary to generate MZBs to be non-haematopoietic [117]. This was followed by an elegant series of experiments whereby *Dll1* and *Dll4* were conditionally inactivated in non-haematopoietic secondary lymphoid organ (SLO) stromal cell populations that expressed a *Ccl19*-driven Cre recombinase [118]. By lineage tracing cells expressing the *Ccl19-Cre* transgene, candidate stromal cell subpopulations were defined among Dll1-expressing cells in the spleen (CD45⁻CD31⁻PDPN⁻) responsible for MZB generation and among Dll4-expressing cells in lymph nodes (CD45⁻CD31⁻CD35^{+/+}-PDPN⁺) necessary for Tfh differentiation. The role for these non-haematopoietic stromal cells in SLOs was particularly intriguing given that the only other known niche for Notch signalling in the thymus similarly depends upon Notch ligand expression by non-haematopoietic cell types (thymic epithelial cells). Past work had also found high levels of Dll1 and Dll4 expression on blood and lymphatic endothelial cell populations [117,119–122]. Endothelial cell populations had been suggested to be the source of Notch ligands in developing liver and neural stem cells [123,124]. Though endothelial cells were also implicated as the ligand source for immune cells [117], more recent work instead points to a non-haematopoietic, non-endothelial cell source [118].

In alloimmunity and GVHD, we found the cellular source of Notch ligands to be non-haematopoietic as well [66]. This finding was unexpected given prior work suggesting that donor and host APCs are critical to activate alloreactive donor T cells in GVHD [125,126]. Using *Ccl19-Cre*-driven *Dll1* and *Dll4* inactivation, we identified a non-haematopoietic SLO stromal cell subpopulation (CD45⁻CD31⁻PDPN⁺CD157⁺) as the likely cellular source of Dll1/Dll4 ligand in GVHD [66]. These fibroblastic stromal cells are believed to reside predominantly in the T zone of the SLO. It is currently unknown why these non-haematopoietic stromal cells are the physiologically relevant source of Notch ligands in allogeneic transplant. It is interesting to speculate that the expression of T-cell chemoattractants CCL19 and CCL21 may render these cells analogous to thymic epithelial cells, which express Dll4 as well as CCL21/25 and CXCL12 under the control of the Foxn1 transcription factor (figure 3) [12]. SLO fibroblastic stromal cells also secrete IL-7, which is necessary for mature T cell survival [127]. Future work should investigate how broadly relevant SLO stromal cells are as a source of Notch ligands in other immunological contexts.

A number of reports have suggested different functional effects of the four agonistic Notch ligands. Data from conditional genetic deletion are limited given uncertainty about the relevant cellular source of ligands. However, work in stromal cells found *Dll1* expression in a splenic stromal cell subpopulation critical for MZB cells, while identifying *Dll4* expression in lymph nodes as essential for Tfh differentiation

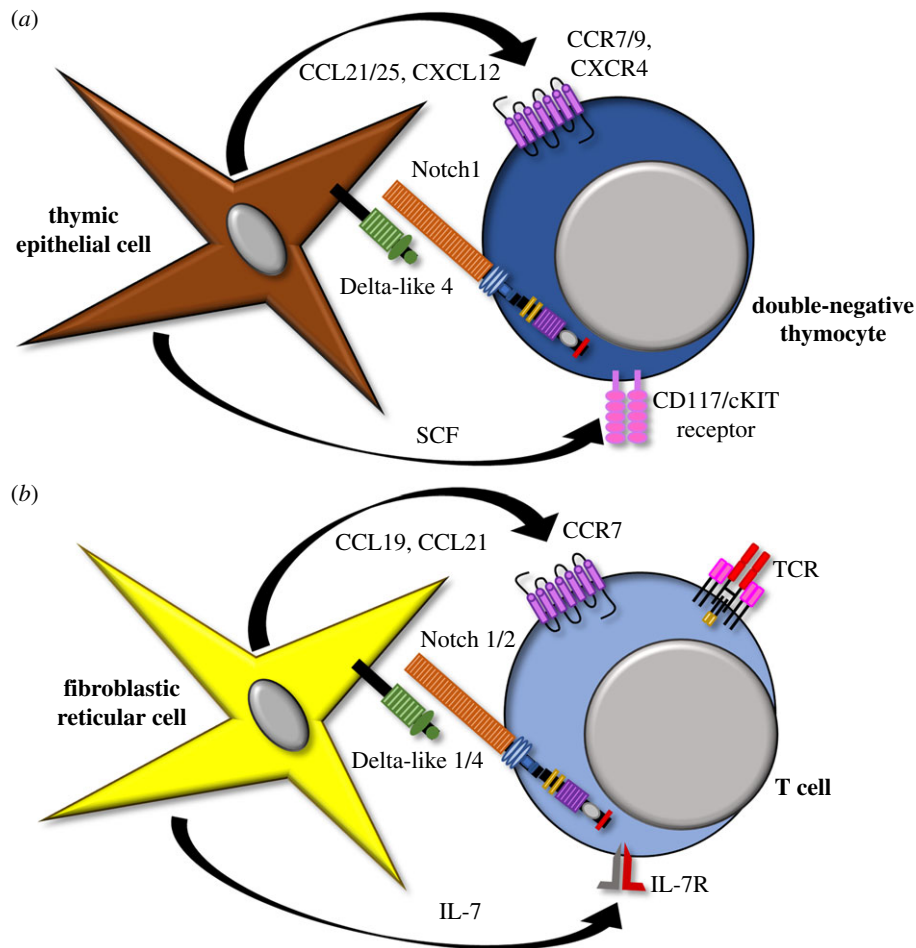


Figure 3. The Notch niche in primary and secondary lymphoid organs. (a) Developing $CD4^-CD8^-$ ('double-negative') thymocytes receive essential Notch signals from Delta-like 4 Notch ligands expressed by thymic epithelial cells. These ligands interact with Notch1 receptors in developing T cells. The specialized thymic niche for T cell development also releases chemokines to attract early T lineage progenitors and Stem Cell Factor (SCF) to support their survival. (b) Mature T cells receive Notch signals from Delta-like 1 and 4 Notch ligands expressed by non-haematopoietic fibroblastic stromal cells in secondary lymphoid organs. These ligands interact with Notch1 and Notch2 expressed by mature T cells. This niche also provides chemokines to attract circulating T cells in the periphery and an IL-7 pro-survival signal.

[118]. These data fit with our work that found a dominant role for Dll4 over Dll1 in alloreactivity using both blocking antibodies and selective deletion in *Ccl19*-expressing stromal cells [60,66]. Other experiments, relying upon *in vitro* and overexpression models, suggested that different ligands induce distinct patterns of differentiation, with Delta-like family members associated with a Th1 response and Jagged with Th2. However, much of this work could be confounded by TLR agonism, which selectively upregulates Delta-like family members over Jagged, making it unclear how much of the Th1/Th2 differentiation bias was instead due to independent effects of TLR agonism [76,77,128]. Blocking antibodies have also suggested different effects from different ligands. Antibodies blocking Dll1 and Dll4—and not Jagged1—could suppress deleterious effects in a multiple sclerosis mouse model [54,129]. The mechanistic basis for qualitatively different signals from Delta-like and Jagged family members, which both signal similarly through the Notch receptor, is unclear. Indeed, another group using ligand overexpression in APCs did not observe a Delta-like/Jagged bias in Th1/Th2 instruction in the absence of polarizing cytokines [130]. It may be that the seemingly different effects of the different ligands are instead attributable to different cellular sources of ligands. Overall, loss-of-function *in vivo* approaches promise to provide the most reliable and physiologically relevant answers.

8. Concluding remarks

A picture of how Notch contributes to the functions of mature T cells in the periphery is slowly coming into view, although many outstanding questions remain. Importantly, lessons learned from studying the role of Notch in T cell development bore significantly on the study of Notch in mature T cells and will play an important role in future experiments to resolve open questions. Specifically, the value of *in vivo*, loss-of-function experiments in this context cannot be overstated. This was seen during experiments deciphering downstream Notch signalling in T cells, mapping the role of Notch in T cell differentiation and identifying the cellular source of Notch ligands. Furthermore, the concept of Notch as a bimodal switch responsible for binary cell fate decisions had to be revised, both in developing and in mature T cells, to make way for a more nuanced picture of Notch as an amplifier of other activating signals. Finally, recent work suggested that a wider range of candidate cellular sources of ligands should be considered, as non-haematopoietic SLO stromal cells have been convincingly shown to play a role in at least in some settings, even though the bulk of the literature had focused on haematopoietic APCs.

To date, it remains unclear how broadly important stromal cells are as cellular sources of Notch ligands and to what extent

other cells, including haematopoietic APCs, provide Notch signals to T cells in the periphery. Beyond TLR signalling, it is unknown which signalling pathways or transcriptional machinery control the expression of Notch ligands to impact mature T cell function. Characterization of the niche for Notch ligand presentation, as has been done in the thymus for developing T cells, remains to be done. Additionally, the downstream targets of Notch signalling in mature T cells remain ill-defined and insights into how Notch effects gene expression often rely on extrapolation from work in T cell leukaemia and developing thymocytes. Finally, if different Notch ligands do indeed physiologically provoke different functional consequences in mature T cells, the mechanistic basis for this observation remains poorly understood. The answers to

these remaining questions will become particularly relevant in revealing new ways to interfere with the Notch pathway so that it might be targeted therapeutically.

Data accessibility. This article has no additional data.

Authors' contributions. J.D.B and I.M. both contributed to the manuscript writing, revision and figure/table preparation.

Competing interests. The authors have no competing interests to declare.

Funding. Work on Notch signalling in the Maillard laboratory is supported by the National Institute of Allergy and Infectious Diseases (grant no. R01-AI091627 to I.M.) and the Leukemia and Lymphoma Society (grant no. TRP 6583-20 to I.M.).

Acknowledgements. We thank Anneka Allman for assistance with figure design.

References

- Ellisen LW, Bird J, West DC, Soreng AL, Reynolds TC, Smith SD, Sklar J. 1991 TAN-1, the human homolog of the *Drosophila Notch* gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* **66**, 649–661. (doi:10.1016/0092-8674(91)90111-B)
- Weng AP, Ferrando AA, Lee W, Morris JP, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT, Aster JC. 2004 Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* **306**, 269–271. (doi:10.1126/science.1102160)
- Radtke F, Wilson A, Stark G, Bauer M, van Meerwijk J, MacDonald HR, Aguet M. 1999 Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity* **10**, 547–558. (doi:10.1016/S1074-7613(00)80054-0)
- Pui JC *et al.* 1999 Notch1 expression in early lymphopoiesis influences B versus T lineage determination. *Immunity* **11**, 299–308. (doi:10.1016/S1074-7613(00)80105-3)
- Kopan R, Ilagan MXG. 2009 The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell* **137**, 216–233. (doi:10.1016/j.cell.2009.03.045)
- Guruharsha KG, Kankel MW, Artavanis-Tsakonas S. 2012 The Notch signalling system: recent insights into the complexity of a conserved pathway. *Nat. Rev. Genet.* **13**, 654–666. (doi:10.1038/nrg3272)
- Maillard I, Weng AP, Carpenter AC, Rodriguez CG, Sai H, Xu L, Allman D, Aster JC, Pear WS. 2004 Mastermind critically regulates Notch-mediated lymphoid cell fate decisions. *Blood* **104**, 1696–1702. (doi:10.1182/blood-2004-02-0514)
- Nam Y, Sliz P, Song L, Aster JC, Blacklow SC. 2006 Structural basis for cooperativity in recruitment of MAML coactivators to Notch transcription complexes. *Cell* **124**, 973–983. (doi:10.1016/j.cell.2005.12.037)
- Wu L, Aster JC, Blacklow SC, Lake R, Artavanis-Tsakonas S, Griffin JD. 2000 MAML1, a human homologue of *Drosophila* Mastermind, is a transcriptional co-activator for NOTCH receptors. *Nat. Genet.* **26**, 484–489. (doi:10.1038/82644)
- Wang H, Zang C, Taing L, Arnett KL, Wong YJ, Pear WS, Blacklow SC, Liu XS, Aster JC. 2014 NOTCH1-RBPJ complexes drive target gene expression through dynamic interactions with superenhancers. *Proc. Natl Acad. Sci. USA* **111**, 705–710. (doi:10.1073/pnas.1315023111)
- Petrovic J *et al.* 2019 Oncogenic Notch promotes long-range regulatory interactions within hyperconnected 3D cliques. *Mol. Cell* **73**, 1174–1190. (doi:10.1016/j.molcel.2019.01.006)
- Calderón L, Boehm T. 2012 Synergistic, context-dependent, and hierarchical functions of epithelial components in thymic microenvironments. *Cell* **149**, 159–172. (doi:10.1016/j.cell.2012.01.049)
- Hozumi K *et al.* 2008 Delta-like 4 is indispensable in thymic environment specific for T cell development. *J. Exp. Med.* **205**, 2507–2513. (doi:10.1084/jem.20080134)
- Koch U *et al.* 2008 Delta-like 4 is the essential, nonredundant ligand for Notch1 during thymic T cell lineage commitment. *J. Exp. Med.* **205**, 2515–2523. (doi:10.1084/jem.20080829)
- Haines N, Irvine KD. 2003 Glycosylation regulates Notch signalling. *Nat. Rev. Mol. Cell Biol.* **4**, 786–797. (doi:10.1038/nrm1228)
- Moloney DJ *et al.* 2000 Fringe is a glycosyltransferase that modifies Notch. *Nature* **406**, 369–375. (doi:10.1038/35019000)
- Koch U, Lacombe TA, Holland D, Bowman JL, Cohen BL, Egan SE, Guidos CJ. 2001 Subversion of the T/B lineage decision in the thymus by lunatic fringe-mediated inhibition of Notch-1. *Immunity* **15**, 225–236. (doi:10.1016/S1074-7613(01)00189-3)
- O'Neil J *et al.* 2007 FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to γ -secretase inhibitors. *J. Exp. Med.* **204**, 1813–1824. (doi:10.1084/JEM.20070876)
- Thompson BJ, Buonamici S, Sulis ML, Palomero T, Vilimas T, Basso G, Ferrando A, Aifantis I. 2007 The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. *J. Exp. Med.* **204**, 1825–1835. (doi:10.1084/jem.20070872)
- Ryan RJH *et al.* 2017 A B cell regulome links Notch to downstream oncogenic pathways in small B cell lymphomas. *Cell Rep.* **21**, 784–797. (doi:10.1016/j.celrep.2017.09.066)
- Puente XS *et al.* 2011 Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature* **475**, 101–105. (doi:10.1038/nature10113)
- Puente XS *et al.* 2015 Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature* **526**, 519–524. (doi:10.1038/nature14666)
- Bea S *et al.* 2013 Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. *Proc. Natl Acad. Sci. USA* **110**, 18 250–18 255. (doi:10.1073/pnas.1314608110)
- Kiel MJ *et al.* 2012 Whole-genome sequencing identifies recurrent somatic NOTCH2 mutations in splenic marginal zone lymphoma. *J. Exp. Med.* **209**, 1553–1565. (doi:10.1084/jem.20120910)
- Rossi D *et al.* 2012 Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. *Blood* **119**, 521–529. (doi:10.1182/blood-2011-09-379966)
- Kluk MJ *et al.* 2013 Gauging NOTCH1 activation in cancer using immunohistochemistry. *PLoS ONE* **8**, e67306. (doi:10.1371/journal.pone.0067306)
- Fabbri G *et al.* 2017 Common nonmutational NOTCH1 activation in chronic lymphocytic leukemia. *Proc. Natl Acad. Sci. USA* **114**, E2911–E2919. (doi:10.1073/pnas.1702564114)
- Zúñiga-Pflücker JC. 2004 T-cell development made simple. *Nat. Rev. Immunol.* **4**, 67–72. (doi:10.1038/nri1257)
- Radtke F, Fasnacht N, MacDonald HR. 2010 Notch signaling in the immune system. *Immunity* **32**, 14–27. (doi:10.1016/j.immuni.2010.01.004)
- Han H, Tanigaki K, Yamamoto N, Kuroda K, Yoshimoto M, Nakahata T, Ikuta K, Honjo T. 2002 Inducible gene knockout of transcription factor recombination signal binding protein-J reveals its essential role in T versus B lineage decision. *Int. Immunol.* **14**, 637–645. (doi:10.1093/intimm/dfx030)
- Feyerabend TB *et al.* 2009 Deletion of Notch1 converts pro-T cells to dendritic cells and promotes thymic B cells by cell-extrinsic and cell-intrinsic

- mechanisms. *Immunity* **30**, 67–79. (doi:10.1016/j.immuni.2008.10.016)
32. Bell JJ, Bhandoola A. 2008 The earliest thymic progenitors for T cells possess myeloid lineage potential. *Nature* **452**, 764–767. (doi:10.1038/nature06840)
 33. Tikhonova AN *et al.* 2019 The bone marrow microenvironment at single-cell resolution. *Nature* **569**, 222–228. (doi:10.1038/s41586-019-1104-8)
 34. Yu VWC *et al.* 2015 Specific bone cells produce DLL4 to generate thymus-seeding progenitors from bone marrow. *J. Exp. Med.* **212**, 759–774. (doi:10.1084/jem.20141843)
 35. Maeda T *et al.* 2007 Regulation of B versus T lymphoid lineage fate decision by the proto-oncogene LRF. *Science* **316**, 860–866. (doi:10.1126/science.1140881)
 36. Lee S-U *et al.* 2013 LRF-mediated Dll4 repression in erythroblasts is necessary for hematopoietic stem cell maintenance. *Blood* **121**, 918–929. (doi:10.1182/blood-2012-03-418103)
 37. Sambandam A, Maillard I, Zediak VP, Xu L, Gerstein RM, Aster JC, Pear WS, Bhandoola A. 2005 Notch signaling controls the generation and differentiation of early T lineage progenitors. *Nat. Immunol.* **6**, 663–670. (doi:10.1038/ni1216)
 38. Tan JB, Visan I, Yuan JS, Guidos CJ. 2005 Requirement for Notch1 signals at sequential early stages of intrathymic T cell development. *Nat. Immunol.* **6**, 671–679. (doi:10.1038/ni1217)
 39. Wolfer A, Wilson A, Nemir M, MacDonald HR, Radtke F. 2002 Inactivation of Notch1 impairs VDJ β rearrangement and allows pre-TCR-independent survival of early $\alpha\beta$ lineage thymocytes. *Immunity* **16**, 869–879. (doi:10.1016/S1074-7613(02)00330-8)
 40. Maillard I *et al.* 2006 The requirement for Notch signaling at the β -selection checkpoint in vivo is absolute and independent of the pre-T cell receptor. *J. Exp. Med.* **203**, 2239–2245. (doi:10.1084/jem.20061020)
 41. Ciofani M, Zúñiga-Pflücker JC. 2005 Notch promotes survival of pre-T cells at the β -selection checkpoint by regulating cellular metabolism. *Nat. Immunol.* **6**, 881–888. (doi:10.1038/ni1234)
 42. Yashiro-Ohtani Y *et al.* 2009 Pre-TCR signaling inactivates Notch1 transcription by antagonizing E2A. *Genes Dev.* **23**, 1665–1676. (doi:10.1101/gad.1793709)
 43. Wolfer A *et al.* 2001 Inactivation of Notch 1 in immature thymocytes does not perturb CD4 or CD8 T cell development. *Nat. Immunol.* **2**, 235–241. (doi:10.1038/85294)
 44. Tanigaki K, Tsuji M, Yamamoto N, Han H, Tsukada J, Inoue H, Kubo M, Honjo T. 2004 Regulation of $\alpha\beta/\gamma\delta$ T cell lineage commitment and peripheral T cell responses by Notch/RBP-J signaling. *Immunity* **20**, 611–622. (doi:10.1016/S1074-7613(04)00109-8)
 45. Maekawa Y *et al.* 2008 Notch2 integrates signaling by the transcription factors RBP-J and CREB1 to promote T cell cytotoxicity. *Nat. Immunol.* **9**, 1140–1147. (doi:10.1038/ni.1649)
 46. Ito T, Schaller M, Hogaboam CM, Standiford TJ, Sandor M, Lukacs NW, Chensue SW, Kunkel SL. 2008 TLR9 regulates the mycobacteria-elicited pulmonary granulomatous immune response in mice through DC-derived Notch ligand delta-like 4. *J. Clin. Invest.* **119**, 33–46. (doi:10.1172/JCI35647)
 47. Auderset F, Schuster S, Coutaz M, Koch U, Desgranges F, Merck E, MacDonald HR, Radtke F, Tacchini-Cottier F. 2012 Redundant Notch1 and Notch2 signaling is necessary for IFN γ secretion by T helper 1 cells during infection with *Leishmania major*. *PLoS Pathog.* **8**, e1002560. (doi:10.1371/journal.ppat.1002560)
 48. Backer RA *et al.* 2014 A central role for Notch in effector CD8 $^{+}$ T cell differentiation. *Nat. Immunol.* **15**, 1143–1151. (doi:10.1038/ni.3027)
 49. Ito T, Allen RM, Carson WF, Schaller M, Cavassani KA, Hogaboam CM, Lukacs NW, Matsukawa A, Kunkel SL. 2011 The critical role of Notch ligand Delta-like 1 in the pathogenesis of influenza A virus (H1N1) infection. *PLoS Pathog.* **7**, e1002341. (doi:10.1371/journal.ppat.1002341)
 50. Mathieu M, Duval F, Daudelin J-F, Labrecque N. 2015 The Notch signaling pathway controls short-lived effector CD8 $^{+}$ T cell differentiation but is dispensable for memory generation. *J. Immunol.* **194**, 5654–5662. (doi:10.4049/JIMMUNOL.1402837)
 51. Neal LM *et al.* 2017 T cell–restricted Notch signaling contributes to pulmonary Th1 and Th2 immunity during *Cryptococcus neoformans* infection. *J. Immunol.* **199**, 643–655. (doi:10.4049/jimmunol.1601715)
 52. Minter LM *et al.* 2005 Inhibitors of γ -secretase block in vivo and in vitro T helper type 1 polarization by preventing Notch upregulation of Tbx21. *Nat. Immunol.* **6**, 680–688. (doi:10.1038/ni1209x)
 53. Elyaman W, Bassil R, Bradshaw EM, Orent W, Lahoud Y, Zhu B, Radtke F, Yagita H, Khoury SJ. 2012 Notch receptors and Smad3 signaling cooperate in the induction of interleukin-9-producing T cells. *Immunity* **36**, 623–634. (doi:10.1016/j.immuni.2012.01.020)
 54. Bassil R, Zhu B, Lahoud Y, Riella LV, Yagita H, Elyaman W, Khoury SJ. 2011 Notch ligand Delta-like 4 blockade alleviates experimental autoimmune encephalomyelitis by promoting regulatory T cell development. *J. Immunol.* **187**, 2322–2328. (doi:10.4049/jimmunol.1100725)
 55. Jurynczyk M, Jurewicz A, Raine CS, Selmaj K. 2008 Notch3 inhibition in myelin-reactive T cells down-regulates protein kinase C theta and attenuates experimental autoimmune encephalomyelitis. *J. Immunol.* **180**, 2634–2640. (doi:10.4049/jimmunol.180.4.2634)
 56. Jurynczyk M, Jurewicz A, Bielecki B, Raine CS, Selmaj K. 2008 Overcoming failure to repair demyelination in EAE: γ -secretase inhibition of Notch signaling. *J. Neurol. Sci.* **265**, 5–11. (doi:10.1016/j.jns.2007.09.007)
 57. Sandy AR, Stoolman J, Malott K, Pongtorpipat P, Segal BM, Maillard I. 2013 Notch signaling regulates T cell accumulation and function in the central nervous system during experimental autoimmune encephalomyelitis. *J. Immunol.* **191**, 1606–1613. (doi:10.4049/jimmunol.1301116)
 58. Zhang Y *et al.* 2011 Notch signaling is a critical regulator of allogeneic CD4 $^{+}$ T-cell responses mediating graft-versus-host disease. *Blood* **117**, 299–308. (doi:10.1182/blood-2010-03-271940)
 59. Sandy AR *et al.* 2013 T cell-specific Notch inhibition blocks graft-versus-host disease by inducing a hyporesponsive program in alloreactive CD4 $^{+}$ and CD8 $^{+}$ T cells. *J. Immunol.* **190**, 5818–5828. (doi:10.4049/jimmunol.1203452)
 60. Tran IT *et al.* 2013 Blockade of individual Notch ligands and receptors controls graft-versus-host disease. *J. Clin. Invest.* **123**, 1590–1604. (doi:10.1172/JCI65477)
 61. Radojic V *et al.* 2018 Notch signaling mediated by Delta-like ligands 1 and 4 controls the pathogenesis of chronic GVHD in mice. *Blood* **132**, 2188–2200. (doi:10.1182/blood-2018-03-841155)
 62. Roderick JE *et al.* 2013 Therapeutic targeting of NOTCH signaling ameliorates immune-mediated bone marrow failure of aplastic anemia. *J. Exp. Med.* **210**, 1311–1329. (doi:10.1084/jem.20112615)
 63. Mochizuki K *et al.* 2013 Delta-like ligand 4 identifies a previously uncharacterized population of inflammatory dendritic cells that plays important roles in eliciting allogeneic T cell responses in mice. *J. Immunol.* **190**, 3772–3782. (doi:10.4049/jimmunol.1202820)
 64. Charbonnier L-M, Wang S, Georgiev P, Sefik E, Chatila TA. 2015 Control of peripheral tolerance by regulatory T cell–intrinsic Notch signaling. *Nat. Immunol.* **16**, 1162–1173. (doi:10.1038/ni.3288)
 65. Chung J *et al.* 2019 Early Notch signals induce a pathogenic molecular signature during priming of alloantigen-specific conventional CD4 $^{+}$ T cells in graft-versus-host disease. *J. Immunol.* **203**, j1900192. (doi:10.4049/jimmunol.1900192)
 66. Chung J *et al.* 2017 Fibroblastic niches prime T cell alloimmunity through Delta-like Notch ligands. *J. Clin. Invest.* **127**, 1574–1588. (doi:10.1172/JCI89535)
 67. Riella LV *et al.* 2011 Blockade of Notch ligand Delta1 promotes allograft survival by inhibiting alloreactive Th1 cells and cytotoxic T cell generation. *J. Immunol.* **187**, 4629–4638. (doi:10.4049/jimmunol.1004076)
 68. Wood S *et al.* 2015 Transient blockade of delta-like Notch ligands prevents allograft rejection mediated by cellular and humoral mechanisms in a mouse model of heart transplantation. *J. Immunol.* **194**, 2899–2908. (doi:10.4049/jimmunol.1402034)
 69. Magee CN *et al.* 2019 Notch-1 inhibition promotes immune regulation in transplantation via Treg-dependent mechanisms. *Circulation* **140**, 846–863. (doi:10.1161/CIRCULATIONAHA.119.040563)
 70. Laky K, Evans S, Perez-Diez A, Fowlkes B. 2015 Notch signaling regulates antigen sensitivity of naive CD4 $^{+}$ T cells by tuning co-stimulation. *Immunity* **42**, 80–94. (doi:10.1016/j.immuni.2014.12.027)

71. Helbig C *et al.* 2012 Notch controls the magnitude of T helper cell responses by promoting cellular longevity. *Proc. Natl Acad. Sci. USA* **109**, 9041–9046. (doi:10.1073/pnas.1206044109)
72. Hoyne GF *et al.* 2000 *Serrate1*-induced Notch signalling regulates the decision between immunity and tolerance made by peripheral CD4⁺ T cells. *Int. Immunol.* **12**, 177–185. (doi:10.1093/intimm/12.2.177)
73. Yvon ES, Vigouroux S, Rousseau RF, Biagi E, Amrolia P, Dotti G, Wagner H-J, Brenner MK. 2003 Overexpression of the Notch ligand, Jagged-1, induces alloantigen-specific human regulatory T cells. *Blood* **102**, 3815–3821. (doi:10.1182/blood-2002-12-3826)
74. Vigouroux S, Yvon E, Wagner H-J, Biagi E, Dotti G, Sili U, Lira C, Rooney CM, Brenner MK. 2003 Induction of antigen-specific regulatory T cells following overexpression of a Notch ligand by human B lymphocytes. *J. Virol.* **77**, 10 872–10 880. (doi:10.1128/JVI.77.20.10872-10880.2003)
75. Skokos D, Nussenzweig MC. 2007 CD8⁺ DCs induce IL-12-independent Th1 differentiation through Delta 4 Notch-like ligand in response to bacterial LPS. *J. Exp. Med.* **204**, 1525–1531. (doi:10.1084/jem.20062305)
76. Sun J, Krawczyk CJ, Pearce EJ. 2008 Suppression of Th2 cell development by Notch ligands Delta1 and Delta4. *J. Immunol.* **180**, 1655–1661. (doi:10.4049/JIMMUNOL.180.3.1655)
77. Amsen D, Blander JM, Lee GR, Tanigaki K, Honjo T, Flavell RA. 2004 Instruction of distinct CD4 T helper cell fates by different Notch ligands on antigen-presenting cells. *Cell* **117**, 515–526. (doi:10.1016/S0092-8674(04)00451-9)
78. Fakiola M *et al.* 2011 Genetic and functional evidence implicating *DLL1* as the gene that influences susceptibility to visceral leishmaniasis at chromosome 6q27. *J. Infect. Dis.* **204**, 467–477. (doi:10.1093/infdis/jir284)
79. Dees C *et al.* 2011 Inhibition of Notch signaling prevents experimental fibrosis and induces regression of established fibrosis. *Arthritis Rheum.* **63**, 1396–1404. (doi:10.1002/art.30254)
80. Shang Y, Smith S, Hu X. 2016 Role of Notch signaling in regulating innate immunity and inflammation in health and disease. *Protein Cell* **7**, 159–174. (doi:10.1007/s13238-016-0250-0)
81. Zhang W, Xu W, Xiong S. 2010 Blockade of Notch1 signaling alleviates murine lupus via blunting macrophage activation and M2b polarization. *J. Immunol.* **184**, 6465–6478. (doi:10.4049/jimmunol.0904016)
82. Chung J, Riella LV, Maillard I. 2016 Targeting the Notch pathway to prevent rejection. *Am. J. Transplant.* **16**, 3079–3085. (doi:10.1111/ajt.13816)
83. Wong KK *et al.* 2003 Notch ligation by Delta1 inhibits peripheral immune responses to transplantation antigens by a CD8⁺ cell-dependent mechanism. *J. Clin. Invest.* **112**, 1741–1750. (doi:10.1172/JCI18020)
84. Lin Y *et al.* 2015 Overexpression of Jagged-1 combined with blockade of CD40 pathway prolongs allograft survival. *Immunol. Cell Biol.* **93**, 213–217. (doi:10.1038/icb.2014.84)
85. Adler SH, Chiffolleau E, Xu L, Dalton NM, Burg JM, Wells AD, Wolfe MS, Turka LA, Pear WS. 2003 Notch signaling augments T cell responsiveness by enhancing CD25 expression. *J. Immunol.* **171**, 2896–2903. (doi:10.4049/JIMMUNOL.171.6.2896)
86. Hasserjian RP, Aster JC, Davi F, Weinberg DS, Sklar J. 1996 Modulated expression of Notch1 during thymocyte development. *Blood* **88**, 970–976.
87. Ohishi K, Varnum-Finney B, Flowers D, Anasetti C, Myerson D, Bernstein ID. 2000 Monocytes express high amounts of Notch and undergo cytokine specific apoptosis following interaction with the Notch ligand, Delta-1. *Blood* **95**, 2847–2854. (doi:10.1182/blood.V95.9.2847.009k19_2847_2854)
88. Koyanagi A, Sekine C, Yagita H. 2012 Expression of Notch receptors and ligands on immature and mature T cells. *Biochem. Biophys. Res. Commun.* **418**, 799–805. (doi:10.1016/j.bbrc.2012.01.106)
89. Waegemans E *et al.* 2014 Notch3 activation is sufficient but not required for inducing human T-lineage specification. *J. Immunol.* **193**, 5997–6004. (doi:10.4049/jimmunol.1400764)
90. Bernasconi-Elias P *et al.* 2016 Characterization of activating mutations of NOTCH3 in T-cell acute lymphoblastic leukemia and anti-leukemic activity of NOTCH3 inhibitory antibodies. *Oncogene* **35**, 6077–6086. (doi:10.1038/onc.2016.133)
91. Barbarulo A *et al.* 2011 Notch3 and canonical NF- κ B signaling pathways cooperatively regulate Foxp3 transcription. *J. Immunol.* **186**, 6199–6206. (doi:10.4049/jimmunol.1002136)
92. Maekawa Y, Tsukumo S, Chiba S, Hirai H, Hayashi Y, Okada H, Kishihara K, Yasutomo K. 2003 Delta1-Notch3 interactions bias the functional differentiation of activated CD4⁺ T cells. *Immunity* **19**, 549–559. (doi:10.1016/S1074-7613(03)00270-X)
93. Palaga T, Miele L, Golde TE, Osborne BA. 2003 TCR-mediated Notch signaling regulates proliferation and IFN- γ production in peripheral T cells. *J. Immunol.* **171**, 3019–3024. (doi:10.4049/jimmunol.171.6.3019)
94. Fang TC, Yashiro-Ohtani Y, Del Bianco C, Knoblock DM, Blacklow SC, Pear WS. 2007 Notch directly regulates *Gata3* expression during T helper 2 cell differentiation. *Immunity* **27**, 100–110. (doi:10.1016/j.immuni.2007.04.018)
95. Mukherjee S, Schaller MA, Neupane R, Kunkel SL, Lukacs NW. 2009 Regulation of T cell activation by Notch ligand, DLL4, promotes IL-17 production and Rorc activation. *J. Immunol.* **182**, 7381–7388. (doi:10.4049/jimmunol.0804322)
96. Bailis W, Yashiro-Ohtani Y, Fang TC, Hatton RD, Weaver CT, Artis D, Pear WS. 2013 Notch simultaneously orchestrates multiple helper T cell programs independently of cytokine signals. *Immunity* **39**, 148–159. (doi:10.1016/J.IMMUNI.2013.07.006)
97. García-Peydró M, de Yébenes VG, Toribio ML. 2006 Notch1 and IL-7 receptor interplay maintains proliferation of human thymic progenitors while suppressing non-T cell fates. *J. Immunol.* **177**, 3711–3720. (doi:10.4049/jimmunol.177.6.3711)
98. González-García S, García-Peydró M, Martín-Gayo E, Ballestar E, Esteller M, Bornstein R, de la Pompa JL, Ferrando AA, Toribio ML. 2009 CSL–MAML-dependent Notch1 signaling controls T lineage-specific IL-7R α gene expression in early human thymopoiesis and leukemia. *J. Exp. Med.* **206**, 779–791. (doi:10.1084/jem.20081922)
99. González-García S, Mosquera M, Fuentes P, Palumbo T, Escudero A, Pérez-Martínez A, Ramírez M, Corcoran AE, Toribio ML. In press. IL-7R is essential for leukemia-initiating cell activity and pathogenesis of T-cell acute lymphoblastic leukemia. *Blood*, blood.2019000982. (doi:10.1182/blood.2019000982)
100. Minter LM, Osborne BA. 2012 Canonical and non-canonical notch signaling in CD4⁺ T cells. In *Notch regulation of the immune system* (ed. F Radtke), pp. 99–114. Berlin, Germany: Springer.
101. Shin HM *et al.* 2014 NOTCH1 can initiate NF- κ B activation via cytosolic interactions with components of the T cell signalosome. *Front. Immunol.* **5**, 249. (doi:10.3389/fimmu.2014.00249)
102. Shin HM, Minter LM, Cho OH, Gottipati S, Fauq AH, Golde TE, Sonenshein GE, Osborne BA. 2006 Notch1 augments NF- κ B activity by facilitating its nuclear retention. *EMBO J.* **25**, 129–138. (doi:10.1038/sj.emboj.7600902)
103. Martínez AA, Zecchini V, Brennan K. 2002 CSL-independent Notch signalling: a checkpoint in cell fate decisions during development? *Curr. Opin. Genet. Dev.* **12**, 524–533. (doi:10.1016/S0959-437X(02)00336-2)
104. Shawber C, Nofziger D, Hsieh JJ, Lindsell C, Böglér O, Hayward D, Weinmaster G. 1996 Notch signaling inhibits muscle cell differentiation through a CBF1-independent pathway. *Development* **122**, 3765–3773.
105. Szabo SJ, Sullivan BM, Peng SL, Glimcher LH. 2003 Molecular mechanisms regulating TH1 immune responses. *Annu. Rev. Immunol.* **21**, 713–758. (doi:10.1146/annurev.immunol.21.120601.140942)
106. Zheng W, Flavell RA. 1997 The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* **89**, 587–596. (doi:10.1016/S0092-8674(00)80240-8)
107. Zhu J *et al.* 2004 Conditional deletion of Gata3 shows its essential function in TH1–TH2 responses. *Nat. Immunol.* **5**, 1157–1165. (doi:10.1038/ni1128)
108. Iwasaki A, Medzhitov R. 2015 Control of adaptive immunity by the innate immune system. *Nat. Immunol.* **16**, 343–353. (doi:10.1038/ni.3123)
109. Amsen D, Helbig C, Backer RA. 2015 Notch in T cell differentiation: all things considered. *Trends Immunol.* **36**, 802–814. (doi:10.1016/J.IT.2015.10.007)
110. Tu L, Fang TC, Artis D, Shestova O, Pross SE, Maillard I, Pear WS. 2005 Notch signaling is an important regulator of type 2 immunity. *J. Exp. Med.* **202**, 1037–1042. (doi:10.1084/jem.20050923)

111. Crotty S. 2014 T follicular helper cell differentiation, function, and roles in disease. *Immunity* **41**, 529–542. (doi:10.1016/j.immuni.2014.10.004)
112. Auderset F *et al.* 2013 Notch signaling regulates follicular helper T cell differentiation. *J. Immunol.* **191**, 2344–2350. (doi:10.4049/jimmunol.1300643)
113. Dominguez-Villar M, Hafler DA. 2018 Regulatory T cells in autoimmune disease. *Nat. Immunol.* **19**, 665–673. (doi:10.1038/s41590-018-0120-4)
114. Samon JB, Champhekar A, Minter LM, Telfer JC, Miele L, Fauq A, Das P, Golde TE, Osborne BA. 2008 Notch1 and TGF β 1 cooperatively regulate Foxp3 expression and the maintenance of peripheral regulatory T cells. *Blood* **112**, 1813–1821. (doi:10.1182/blood-2008-03-144980)
115. Yamaguchi E, Chiba S, Kumano K, Kunisato A, Takahashi T, Takahashi T, Hirai H. 2002 Expression of Notch ligands, Jagged1, 2 and Delta1 in antigen presenting cells in mice. *Immunol. Lett.* **81**, 59–64. (doi:10.1016/S0165-2478(01)00326-1)
116. Schaller MA, Neupane R, Rudd BD, Kunkel SL, Kallal LE, Lincoln P, Lowe JB, Man Y, Lukacs NW. 2007 Notch ligand Delta-like 4 regulates disease pathogenesis during respiratory viral infections by modulating Th2 cytokines. *J. Exp. Med.* **204**, 2925–2934. (doi:10.1084/jem.20070661)
117. Tan JB, Xu K, Cretegnny K, Visan I, Yuan JS, Egan SE, Guidos CJ. 2009 Lunatic and manic fringe cooperatively enhance marginal zone B cell precursor competition for Delta-like 1 in splenic endothelial niches. *Immunity* **30**, 254–263. (doi:10.1016/j.immuni.2008.12.016)
118. Fasnacht N *et al.* 2014 Specific fibroblastic niches in secondary lymphoid organs orchestrate distinct Notch-regulated immune responses. *J. Exp. Med.* **211**, 2265–2279. (doi:10.1084/jem.20132528)
119. Shutter JR, Scully S, Fan W, Richards WG, Kitajewski J, Deblandre GA, Kintner CR, Stark KL. 2000 Dll4, a novel Notch ligand expressed in arterial endothelium. *Genes Dev.* **14**, 1313–1318.
120. Hellström M *et al.* 2007 Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. *Nature* **445**, 776–780. (doi:10.1038/nature05571)
121. Zheng W *et al.* 2011 Notch restricts lymphatic vessel sprouting induced by vascular endothelial growth factor. *Blood* **118**, 1154–1162. (doi:10.1182/blood-2010-11-317800)
122. Niessen K, Zhang G, Ridgway JB, Chen H, Kolumam G, Siebel CW, Yan M. 2011 The Notch1-Dll4 signaling pathway regulates mouse postnatal lymphatic development. *Blood* **118**, 1989–1997. (doi:10.1182/blood-2010-11-319129)
123. McCright B, Lozier J, Gridley T. 2002 A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency. *Development* **129**, 1075–1082.
124. Shen Q, Goderie SK, Jin L, Karanth N, Sun Y, Abramova N, Vincent P, Pumiglia K, Temple S. 2004 Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* **304**, 1338–1340. (doi:10.1126/SCIENCE.1095505)
125. Magenau J, Runaas L, Reddy P. 2016 Advances in understanding the pathogenesis of graft-versus-host disease. *Br. J. Haematol.* **173**, 190–205. (doi:10.1111/bjh.13959)
126. Blazar BR, Murphy WJ, Abedi M. 2012 Advances in graft-versus-host disease biology and therapy. *Nat. Rev. Immunol.* **12**, 443–458. (doi:10.1038/nri3212)
127. Link A, Vogt TK, Favre S, Britschgi MR, Acha-Orbea H, Hinz B, Cyster JG, Luther SA. 2007 Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat. Immunol.* **8**, 1255–1265. (doi:10.1038/ni1513)
128. Napolitani G, Rinaldi A, Bertoni F, Sallusto F, Lanzavecchia A. 2005 Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat. Immunol.* **6**, 769–776. (doi:10.1038/ni1223)
129. Elyaman W, Bradshaw EM, Wang Y, Oukka M, Kivisäkk P, Chiba S, Yagita H, Khoury SJ. 2007 Jagged1 and Delta1 differentially regulate the outcome of experimental autoimmune encephalomyelitis. *J. Immunol.* **179**, 5990–5998. (doi:10.4049/JIMMUNOL.179.9.5990)
130. Ong C-T, Sedy JR, Murphy KM, Kopan R. 2008 Notch and presenilin regulate cellular expansion and cytokine secretion but cannot instruct Th1/Th2 fate acquisition. *PLoS ONE* **3**, e2823. (doi:10.1371/journal.pone.0002823)