



Review

Recent Advances in $\alpha\beta$ T Cell Biology: Wnt Signaling, Notch Signaling, Hedgehog Signaling and Their Translational Perspective

Wenping Lin ¹, Kai Dai ², Luokun Xie ^{3, *}

¹ Department of Orthopedic Surgery, the Second Affiliated Hospital, Fujian Medical University, Quanzhou, China;

² Department of Infectious Diseases, Renmin Hospital of Wuhan University, Wuhan, China;

³ University of North Texas Health Science Center, Fort Worth, Texas, USA

* **Correspondence:** Email: luokun.xie@unthsc.edu; Tel: 817-735-2766

Abstract: Engagement of bioactive ligands with cell surface receptors plays critical roles in the initiation and regulation of $\alpha\beta$ T cell development, homeostasis and functions. In the past two decades, new subpopulations of $\alpha\beta$ T cells have been discovered. In addition, the characterization of new ligand/receptor axes has led to a better understanding of $\alpha\beta$ T cell biology. In the current review, the phenotypic and functional properties of $\alpha\beta$ T cell subpopulations are described, as well as the effects of three novel and well-documented signal pathways—Wnt, Notch and Hedgehog signaling—on $\alpha\beta$ T cell development and functions are summarized. These signal pathways are initiated by the ligation of corresponding ligands with respective receptors, and this subsequently exerts a positive or negative influence on $\alpha\beta$ T cell ontogenesis and behavior. Thorough understanding of the components of these signal pathways might shed new light on the manipulation of $\alpha\beta$ T cell biology so as to favor the advance of diagnosis and therapy of immune disorders such as infection, tumors and autoimmune diseases.

Keywords: $\alpha\beta$ T cells; Cell signaling; Wnt; Notch; Hedgehog

Wenping Lin and Kai Dai contributed equally to this review.

Abbreviations

CD: cluster of differentiation; IL: interleukin; IFN: interferon; TGF: transforming growth factor; LT: lymphotoxin; TCR: T cell receptor

1. Introduction

Immune cells interact with each other and target cells through direct and indirect contact to maintain survival, initiate immune responses, transmigrate to target sites and launch immune attacks or immunoregulation. Both direct and indirect cell-cell contact involve engagement of bioactive ligands and receptors. As a critical component of cellular immune responses, $\alpha\beta$ T cells express a complicated array of receptors and ligands to satisfy the demand of accurate and timely reaction against foreign pathogens and mutated self-antigens. Classical ligands that regulate $\alpha\beta$ T cell development and function, such as MHC molecules, co-stimulatory molecules, cytokines, chemokines and adhesion molecules, have been extensively studied and greatly favor the advance of diagnostic and therapeutic approaches for various diseases. Importantly, in the past decades, newly discovered ligand/receptor axes that play significant parts in the shaping and modulation of T cell immunity have substantially deepened our understanding of the immune system. In this review, we focus on three well-documented signal pathways induced by novel ligand/receptor axes—Wnt signaling, Notch signaling and Hedgehog signaling and summarize their roles in $\alpha\beta$ T cell development and functions, with subsequent discussion of the potential mechanisms underlying their effects.

2. T cells

T cells are the cellular components of adaptive immunity in the body. Originated from hematopoietic stem cells and developing in the thymus, each T cell targets a unique antigen epitope with their specified antigen recognition receptors on their surface [1]. T cells can be grouped into various subsets based on their effector functions and molecular phenotype. Distinct T cell subsets promote different types of immune response. About 95% of T cells are $\alpha\beta$ T cells, named after the T cell receptor (TCR) consisting of an α and a β chain [2]. The thymus is the main producer of $\alpha\beta$ T cells. $\alpha\beta$ T cells exit from the thymus to enter the blood circulation and home to secondary lymphoid organ/tissues such as spleen, lymph nodes, tonsils, Peyer's patches and mucosa associated lymphoid tissue (MALT) [3]. Based on the expression of surface CD4 and CD8 glycoprotein, $\alpha\beta$ T cells can be further divided into CD4⁺ and CD8⁺ T cells.

2.1. CD4⁺ T cells

Also known as helper T cells, CD4⁺ T cells are crucial in achieving effective adaptive immune response to pathogens. Naive CD4⁺ T cells are activated after interaction with antigen-MHC complex

presented by antigen presenting cells (APCs) and differentiate into specific subtypes depending on the cytokine milieu of the microenvironment [4]. CD4⁺ T cells help B cells make antibody, enhance and maintain responses of CD8⁺ T cells, and regulate macrophage function. In addition, CD4⁺ T cells play critical role in immunologic memory [5]. CD4⁺ T cells can be activated in response to a particular cytokine milieu and may differentiate into one of several lineages, including Th1, Th2, Th17, Th9, Treg, and T_{FH}, as defined by their pattern of cytokines production and function [5]. Table 1 summarizes the differentiation and functions of the major CD4⁺ T cell subtypes.

Table 1. CD4⁺ T cell subtypes.

Subtype	Inducing cytokine	Master regulator	Effector Cytokine	Function
Th1	IL-12, IFN- γ	T-bet, STAT1	IFN- γ , LT α / β	Anti-viral and anti-bacterial immunity
Th2	IL-4, IL-2	GATA-3, STAT6	IL-4, IL-5, IL-9, IL-10, IL-13	Extracellular parasites immunity
Th17	TGF- β , IL-6, IL-21, IL-23	ROR γ t, STAT3	IL-17a, IL-21, IL-22	Inflammation, autoimmunity
Th9	TGF- β , IL-4	IRF4*	IL-9	Inflammation, autoimmunity, anti-tumor immunity
Treg	TGF- β	Foxp3	IL-10, TGF- β , IL-35	Anti-inflammation, Anti-autoimmunity
T_{FH}	IL-6, IL-21	STAT3*	IL-10, IL-21, IL-4	Help B cell differentiation

* Important but might not be the master regulator.

2.2. CD8⁺ T cells

CD8⁺ T cells are the major fighters against viral infections but also participate in defense against bacterial and protozoal infections [6]. Resting naive CD8⁺ T cells react to pathogens by massive expansion and differentiation into cytotoxic effector cells that migrate to all regions of the body to clear the infection. Pro-inflammatory cytokines, such as IL-12, play a key role in terminal differentiation of CD8⁺ effector T cells [7,8]. CTLs are equipped with effector agents such as IFN- γ , TNF- α , perforin, granzyme B and Fas ligand to kill pathogen-infected or dysfunctional somatic cells.

3. Wnt Signaling

Wnt proteins are a family of 19 secreted glycoproteins that have crucial roles in the regulation of diverse processes, including cell proliferation, survival, migration and polarity, specification of cell fate, and self-renewal in stem cells and tumor cells [9,10]. The detailed characterization of canonical and non-canonical Wnt signal pathways, together with their significance in diverse diseases, have been well documented in previous reviews [11–13]. Notably, numerous reports have

disclosed the role of Wnt signaling in the development and function of T cells, emphasizing the importance of Wnt proteins for T cell immunity. In the following we summarized the recent findings in this area.

3.1. *Wnt and T cell development*

Wnt proteins and related signal transduction have been known to influence lymphocyte development [14]. T cells are generated in the thymus, and thymic epithelium is the main source of Wnt proteins [15,16]. Expression of ecto-domains of Frizzled receptors by retroviral transduction as extracellular inhibitors of Wnt signaling in fetal thymus organ culture induces a complete block of early thymocyte development, indicating that secreted Wnt factors are essential for intrathymic T lineage development [17]. Specifically, expression of Wnt1 and Wnt4 through retroviral transduction yielded increased numbers of cultured thymocytes in the absence of stroma, likely through a combined effect on survival and proliferation [17]. However, the role of Wnt signaling might be dependent on development phases. Stabilization of β -catenin in thymocytes leads to the generation of double positive thymocytes lacking surface expression of the TCR/CD3 complex, indicating that the effects of stabilized β -catenin occurred independent of, or parallel to, pre-TCR signaling and TCR selection in double negative thymocytes [18]. Indeed, it has been shown that Wnt signaling occurs in the thymus and is most active in the immature double negative stages [19]. Differential sensitivity to Wnt signaling at different thymocyte stages was not caused by increased expression of Frizzled or Wnt proteins on the most immature thymocytes, as Frizzled and Wnt seem to be expressed at comparable levels in all thymocyte subsets. Instead, increased expression of positively acting canonical Wnt factors (such as β -catenin) and decreased expression of inhibitory molecules (such as AXIN1) was observed in double negative thymocytes [19]. So, responsiveness to Wnt signaling in thymocytes appears to be regulated by the differential expression of intracellular signal-transduction molecules. In addition, other studies also illustrate the importance of Wnt signaling in T cell development. Conditional T-cell-specific deletion of β -catenin, using the proximal LCK promoter to control Cre expression, impaired T-cell development at the β -selection checkpoint, leading to a marked decrease in the number of peripheral T cells [20]. Activation of the WNT pathway by overexpressing activated forms of β -catenin led to the generation of more thymocytes [21].

3.2. *Wnt signaling regulates CD4⁺ T cell functions*

The significance of Wnt signaling for T cell immunity has been studied for the past decade. Once T cells have entered the circulation, they continue to express Wnt receptors Frizzled and LRP5/6, and expression of Frizzled and LRP5/6 are markedly up-regulated on effector T cells [22]. Moreover, the transcription factors involved in Wnt signaling -LEF1 and TCF1- are also dynamically expressed in T cells, with changing protein levels depending on the activation status of T cells [23]. However, the peripheral sources of Wnt proteins, especially in the T cell-mediated immune

responses, have not been completely identified. Most studies use transgenic animal models or chemical compounds to increase or decrease Wnt signaling components to demonstrate the role of Wnt pathway in T cell activity. Interestingly, a recent study revealed T cell's ability to express Wnt7a upon TCR activation and IL-2 stimulation, and postulating that Wnt7a keeps T cells in a non-proliferative state [24].

The impact of Wnt signaling on CD4⁺ T cell behavior has been partially elucidated. It is very likely that the effect of Wnt signaling is differential in distinct subpopulations and functional phases of CD4⁺ T cells. Despite the CD4⁺ T cell subsets, the inhibitory effect of Wnt signaling on CD4⁺ T cell function has been repeatedly observed [25–27]. Wnt signaling initiates the Th2 fate by inducing the transcription factor Gata-3 and repressing IFN- γ [28,29]. However, another study stated that β -catenin is dispensable for T cell effector differentiation, memory formation and recall responses [30]. Interestingly, studies showed that Wnt signaling extends regulatory T cell (Treg) survival [31] but negatively modulates Treg cell function [26]. Moreover, it was reported that Wnt signaling favors Th17 differentiation by up-regulating IL-17a and ROR γ t expression in activated CD4⁺ T cells [27,32]. However, other studies drew the opposite conclusion regarding the Th17 differentiation [33,34]. Therefore, further studies are required to clarify the role of Wnt signaling in CD4⁺ T cell differentiation in distinct scenarios.

3.3. *Wnt signaling regulates CD8⁺ T cell functions*

By using an inhibitor to block the activity of GSK-3 β which acts as a switch in regulating β -catenin stability, it is proposed that Wnt signaling blocks CD8⁺ T cell proliferation and effector differentiation while promoting the generation of memory CD8⁺ T cells [35]. This finding has been supported by subsequent studies indicating the essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8⁺ T cell memory, as well as constitutive activation of Wnt signaling in favor of generation of memory CD8⁺ T cells [36,37]. However, another study argued that β -catenin is not required for the effect of GSK-3 β inhibition on alterations in memory phenotype of CD8⁺ T cells, and even high concentrations of Wnt3a failed to induce β -catenin accumulation in primary T cells [38]. The discrepancy of the above studies emphasizes the urgent need for detailed characterization of Wnt signaling in CD8⁺ T cells. Regardless of this dispute, it is generally considered that active Wnt signaling can keep CD8⁺ T cells in a more undifferentiated state. In addition, the extent of Wnt signaling is also associated with the memory CD8⁺ T cells' function, as demonstrated by increased recall expansion in memory cells with high Wnt levels [39]. Furthermore, a recent study stated that CD8⁺ T cells entering the CNS during HIV infection can give rise to CD4(dim)CD8(bright) T cells through a Wnt signaling-dependent manner, suggesting the importance of Wnt signaling in anti-HIV control in the CNS [40].

4. Notch Signaling

Notch signaling links the fate of one cell to that of an immediate neighbor and consequently controls differentiation, proliferation and apoptotic events in multiple metazoan tissues. The canonical model requires the activation of the Notch receptor through a series of proteolytic events on binding to any of the Delta-Serrate-LAG2 (DSL) ligands. The crucial cleavage event for signaling depends on the γ -secretase-mediated release of the Notch intracellular domain (NICD) from the cell membrane. This allows the translocation of NICD into the nucleus, where it directly participates in a core transcriptional complex with the DNA-binding protein Suppressor of Hairless (SU(H)) and the nuclear effector Mastermind (MAM), thereby activating transcription of target genes. The role of Notch signaling in T cell development and function has been investigated for over two decades. Intriguing discoveries with regard to Notch signaling in T cells are summarized in the following sections.

4.1. *Notch and T cell development*

Notch signaling is highest in immature $\alpha\beta$ T cells (including in early thymic progenitors (ETPs), double-negative 2 (DN2) thymocytes and DN3a thymocytes up to the DN3 stage [41]). In these immature thymocytes, Notch1 is continuously required to restrict developing $\alpha\beta$ T cells to the T cell lineage [42]. Notch1 ligand Delta-like 4 is expressed on thymic epithelial cells and is the essential, non-redundant ligand for Notch1 during thymic T cell lineage commitment [43]. Inactivation of Delta-like 4 in TECs led to a complete block in T cell development [43]. Furthermore, it is reported that other Notch ligands, such as Jagged-1, Jagged-2 and Delta-like-1, are also expressed by thymic epithelium [44,45]. It has been suggested that Jagged2, like Delta-like-1 and Delta-like-4 but in contrast to Jagged1, can mediate the T- versus B-cell lineage decision [45]. Interestingly, the differential effects of Notch ligands on T cell development are also supported by an independent study showing that Delta-1, but not Jagged-1, permits the emergence of a de novo thymocyte population co-expressing CD4 and CD8 [46]. After thymocytes successfully pass β -selection, they immediately down-regulate Notch1 expression.

4.2. *Notch signaling and CD4⁺ T cell functions*

In an in vivo model, administration of γ -secretase inhibitors, which impedes cleavage of Notch, substantially impeded Th1-mediated disease progression by preventing Notch-induced up-regulation of Tbx21 in the mouse experimental autoimmune encephalomyelitis model of multiple sclerosis [47]. However, γ -secretase inhibition may not only affect Notch cleavage but may also affect the cleavage of many other targets that could affect Th cell differentiation independently of Notch signaling, indicating the necessity of specific Notch blockage/knockout in further study. Indeed, in the EAE model, specific induction of Notch1 and Notch3 transcripts were noted in EAE-reactive T cells, and

selective inhibition of Notch3, but not Notch1, receptor abrogated proliferation, Th1- and Th17-type responses [48]. In other research, expression of Notch1 and Notch2 on T cells is crucial for the differentiation of functional IFN- γ -secreting Th1 cells during infection with *L. major* [49].

Differentiation of other Th cells is also influenced by Notch signaling. It is shown that Notch binds to the promoter of GATA3, a master regulator of Th2 differentiation, thus inducing the expression of the GATA3 [50]. The Notch signaling pathway was also shown to cooperate with TGF β to induce Th9 cells. Notch1 intracellular domain recruited Smad3, and together with recombining binding protein (RBP)-J κ bound the Il9 promoter and induced its transactivation [51]. However, a recent research provides a detailed mechanistic study investigating the role of Notch in orchestrating Th cell differentiation. In this experiment, Notch simultaneously regulated the Th1, Th2, and Th17 cell genetic programs independently of cytokine signals, acting as an unbiased amplifier of Th cell differentiation. This raises the question that perhaps Notch signaling equivalently initiates multiple Th programs rather than supporting any specific Th differentiation alone. This hypothesis needs further investigation in distinct disease models [52].

Previous publications accumulated evidence of Notch signaling in Treg cells. It is reported that Notch3 is expressed at a higher level on CD4⁺ CD25⁺ T cells which contain Treg cells [53]. Enforced expression of constitutively active Notch3 in T cells induces the accumulation of Treg cells in the thymus and periphery, leading to protection against autoimmune disorders [53]. Further studies reported that Notch3 also enhanced their suppressive activity by increasing Foxp3 expression [54,55]. These results indicate that Notch signaling favors Treg cell generation and suppressive activity. However, in a recent study using a lineage-specific deletion of components of the Notch pathway, Notch signaling is found to be detrimental for Treg cell-mediated suppression of Th1 responses and protected against their Th1 skewing and apoptosis [56]. In contrast, expression in Treg cells of a gain-of-function transgene encoding the Notch1 intracellular domain resulted in lymphoproliferation, exacerbated Th1 responses and autoimmunity [56]. This study also demonstrated that cell-intrinsic canonical Notch signaling impaired Treg cell fitness and promoted the acquisition by Treg cells of a Th1 cell-like phenotype, whereas non-canonical Notch signaling dependent on the adaptor Rictor activated the kinase AKT-transcription factor Foxo1 axis and impaired the epigenetic stability of Foxp3 [56]. The contradictory data with regard to Notch signaling in Treg cells presents the possibility that different Notch receptors might function differentially, depending on the immune milieu and intensity of Notch signaling. Interestingly, it is reported that Notch activation in effector T cells increases their sensitivity to Treg cell-mediated suppression through up-regulation of TGF- β RII expression [57].

4.3. *Notch signaling and CD8⁺ T cell functions*

The engagement of Notch ligands was shown to regulate the CD8⁺ T cell functions. Ligation of Delta-like 1 to Notch on splenic CD8⁺ cells results in a dramatic decrease in IFN- γ with a concomitant enhancement of IL-10 production in a transplantation model, suggesting that Notch

signaling can alter the differentiation potential of CD8⁺ cells[58]. In addition, administration of anti-Delta-like 1 mAb decreases granzyme B-producing cytotoxic T cells in a mouse cardiac transplant model, further illustrating the importance of Notch signaling for CD8⁺ T cell reaction[59]. Furthermore, Notch2-deficient T cells had impaired differentiation into cytotoxic T lymphocytes, and dendritic cells with lower expression of the Notch ligand Delta-like 1 induces the differentiation of cytotoxic T cells less efficiently both *in vitro* and *in vivo*, demonstrating a crucial role for Notch2 signaling in CD8⁺ T cell cytotoxic responses [60]. This study proves that the intracellular domain of Notch2 interacts with a phosphorylated form of the transcription factor CREB1 to induce granzyme B expression in CD8⁺ T cells [60]. The same group later reported that Notch2 signaling, but not Notch1 signaling, is required for the generation of anti-tumor CD8⁺ T cell responses [61]. Independent research revealed that both Notch1 and Notch2 are critical for the proliferation and IFN- γ production of activated CD8⁺ T cells and are significantly decreased in tumor-infiltrating T cells [62]. Conditional transgenic expression of the Notch1 intracellular domain in antigen-specific CD8⁺ T cells increased both cytotoxicity effects and granzyme B level [62].

Recently, it was found that Notch receptor favors the differentiation of activated CD8⁺ T cells towards terminal effector cells (TEC) instead of memory precursor cells (MPC). In this report, Notch promotes the differentiation of immediately protective TEC and is correspondingly required for the clearance of acute infection with influenza virus [63]. The mechanism under this effect is that Notch activated a major portion of the TEC-specific gene-expression program while suppressing the MPC-specific program. Expression of Notch was induced on naive CD8⁺ T cells by inflammatory mediators and IL-2 via pathways dependent on the metabolic checkpoint kinase mTOR and the transcription factor T-bet. This study is supported by further evidence showing that Notch signaling pathway plays a context-dependent role for optimal cytokine production by effector CD8⁺ T cells in a *Listeria* infection model[64].

5. Hedgehog Signaling

The Hedgehog (Hh) family of proteins control cell growth, survival, and fate, and pattern almost every aspect of the vertebrate body plan. Three mammalian hedgehog proteins have been found, including sonic hedgehog (SHH), Indian hedgehog (IHH) and desert hedgehog (DHH). Hedgehog proteins bind to their receptors, including Ptc, Smo and several co-receptors, to induce signal cascades so as to exert their effects. The detailed summary of Hedgehog proteins and corresponding receptors can be found in previous reviews [65–67]. Here we focus on the effect of Hedgehog signaling in T cell development and functions.

5.1. Hedgehog and T cell development

Like Wnt and Notch ligands, SHH, IHH and DHH are expressed by the thymic epithelial cells in both mice and human thyme [68–71]. In human thymus, SHH-expressing epithelial cells are

restricted to the thymic subcapsula and medulla, whereas IHH- and DHH-producing epithelial cells are distributed throughout the thymus. The requisite Hh receptors, Ptc1 and Smo, and the Gli transcription factors are expressed by thymocytes and also by epithelial cells [71]. In mice, Smo is highest in the DN2 population and is gradually downregulated in the subsequent DN3 and DN4 populations, to be virtually undetectable at the DP stages [68,70]. In SP thymocytes, only 9% of CD4⁺ thymocytes and 20% of CD8⁺ thymocytes express detectable Smo [68]. Consistently, transcription of the hedgehog target gene and hedgehog-responsive-transcriptional activator Gli1 is highest in the DN2 population [70]. Among the three Hh proteins, SHH has been the best characterized in T cell biology until recently. Shh^{-/-}-thymus contains 10 times less thymocytes than the wild type thymus, and has reduced thymocyte proliferation as well as a partial arrest in development at the DN1 stage [69]. Gli3^{-/-}-thymus, which lacks the Gli3 protein downstream of SHH pathway, also has a partial thymocyte arrest at the DN1 stage [72]. Therefore, SHH is essential for differentiation into DN2 thymocytes. However, the function of Hh signaling at the transition from DN to DP cells is controversial because contradictory data has been generated from independent studies, indicating the necessity of more specific and more elaborated studies for complete elucidation of the role of SHH in thymic T cell development.

5.2. *Hedgehog signaling and CD4⁺ T cell function*

Literature has reported Shh and its receptor Ptc are expressed on resting and activated human peripheral CD4⁺ T cells. Addition of exogenous SHH amplified the production of IL-2 and IFN- γ by activated CD4⁺ T cells. Cell surface expression of CD25 and CD69 on activated T cells was up-regulated by exogenous SHH [73]. In addition, exogenous SHH is able to promote entry of T cells into the S-G(2) proliferative phase of the cell cycle, thus enhancing the clonal expansion of T cells [74]. However, it has also been observed that constitutive activation of SHH signaling inhibits T-cell activation and proliferation, most probably by modulating the strength of the TCR signal [75]. The suppressive effect of Hh is supported by the observation that Gli2 transcriptional activator impairs the TCR-induced calcium flux and nuclear expression of NFAT, suppresses up-regulation of molecules essential for activation, and attenuates signaling pathways upstream of AP-1 and NF κ B, leading to reduced activation of CD4⁺ T cells [76]. Surprisingly, conditional deletion of Smo from T cells has failed to reveal any positive or negative influences on agonistic antibody-induced T cell proliferation in vitro [70]. Similarly, an investigation reported that peripheral CD4⁺ T cells were not affected by selective deletion of Ptc gene in CD4⁺ T cells, because proliferation and IFN- γ secretion by *Ptc*-deficient T cells were indistinguishable from controls irrespective of strong or suboptimal stimulatory condition [77]. The controversy of Hh effects on CD4⁺ T cells might reflect a high degree of complexity in Hh signaling in CD4⁺ T cells.

It seems that Hh is not important for Treg function, because Ptc deletion in CD4⁺ T cells does not alter the frequency of Tregs, Foxp3 expression in Tregs, and the immunosuppressive activity of Tregs [77].

5.3. *Hedgehog signaling and CD8⁺ T cell function*

Unfortunately, the role of Hh signaling in the regulation of CD8⁺ T cell function has not been extensively studied. Recent research has presented clues to how Hh impacts CD8⁺ T cell reaction. In 2013, it was discovered that Hh signaling controls T cell killing at the immunological synapse [78]. In this study, TCR activation triggers Hh signaling and expression of Hh components in CD8⁺ T cells. Importantly, IHH, not SHH or DHH, is expressed in both naïve CD8⁺ T cells and cytotoxic T lymphocytes derived from CD8⁺ T cells (CTLs). *Smo* deletion CTLs attenuated target cell killing by CTLs, suggesting Hh signaling promotes CTL-mediated cytotoxicity. The authors proposed that Hh signaling prearms CTLs with the ability to rapidly polarize the cytoskeleton and deliver the cytotoxic granules within minutes when CTLs encounter targets. Thus, it is likely that Hh signaling facilitates the release cytotoxic mediators from CTLs rather than altering the expression of cytotoxic mediators.

6. **Perspective**

In the past two decades, discovery of signal pathways induced by new ligand-receptor axis on T cells has led to deeper understanding of the immune response and regulation. Wnt signaling, Notch signaling and Hh signaling are just three representatives among those exciting findings. Although these signal pathways were originally characterized in stem cell biology, their significance for mature T cell function suggests that more stem cell-related signaling might be functioning in T cells at different stages, including naïve resting stage, activation stage, effector stage and memory stage.

Therapeutic strategies targeting the above three signaling systems in T cells have been proposed. The crucial role of Wnt signaling in inducing and maintaining memory T cells, especially memory CD8⁺ T cells, has sparked researchers' interest in its potential application in cancer therapy. Inhibition of Gsk-3 β , which promotes Wnt signaling, has been shown to enhance memory T cell generation in response to a tumor antigen [35]. Wnt signaling could also be a target in rheumatoid arthritis therapy, since CD4⁺ T cells in rheumatoid arthritis patients have an aberrant regulation of Wnt signaling [79]. If further investigation reveals the role of Wnt signaling in autoreactive T cells, new therapies might be designed for autoimmune diseases including rheumatoid arthritis and multiple sclerosis. In addition, inhibition of Wnt signaling in Treg cells might promote the immunosuppressive activity of Treg cells, which would be beneficial for therapies against autoimmune diseases.

The critical function of Notch signaling in T cell development makes it a potential target for T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) therapy. Activating *Nothc1* mutations were identified in over 60% of T-ALLs [80]. High *Nothc1* signaling deregulates other signal pathways which are important for cell growth and differentiation. Thus, application of γ -secretase inhibitors, which abrogate Notch signaling, become an emerging target therapy for T-ALL [81]. Additionally, the role of Notch signaling in helper T cell differentiation has shed new light on autoimmune disorder therapies. γ -secretase inhibitors reduces Th1- and Th17-mediated inflammatory responses in

a mouse model of rheumatoid arthritis [82]. Administering soluble Jag1 provides a negative signal to CD8⁺ T cells and reduces disease symptoms in a rheumatoid arthritis model [83]. In systemic lupus erythematosus patients, down-regulated Notch1 signaling is associated with decreased generation and proliferation of Treg cells [84], as well as increase in IL-17-producing T cells [85]. Therefore, augmentation of Notch1 signaling in Treg cells could slow the progression of systemic lupus erythematosus. Furthermore, γ -secretase inhibitors significantly decrease Th1 and Th17 cytokines in an experimental autoimmune encephalomyelitis model [48]. Delta-like 4 blockade alleviates the clinical severity and shifts the immune balance from a Th1/Th17 mediated response toward a Th2/Treg mediated response [86]. All of the above studies demonstrate promising therapies which manipulate Notch signaling in T cells to treat autoimmune disorders.

With regard to Hh signaling in T cells, the research on its translational potential is scarce. This is partially due to the lack of hard evidence showing the importance of Hh signaling for T cell function. It has been revealed that Hh signaling is activated in a subset of T-ALL and T-lymphoblastic lymphoma (T-LBL) through mutations in critical proteins of the pathway [87,88]. Future investigation will be required to demonstrate the significance or insignificance of Hh signaling in T cell-mediated diseases.

Through dissection and analysis of these ligand-receptor axes and their interplay with other known signal pathways novel approaches can conceivably be designed for the diagnosis and treatment of infectious, autoimmune and tumorigenic disorders

Conflict of Interest

The authors declare no conflict of interest.

References

1. Reiner SL (2009) Decision making during the conception and career of CD4⁺ T cells. *Nat Rev Immunol* 9: 81-82.
2. Clambey ET, Davenport B, Kappler JW, et al. (2014) Molecules in medicine mini review: the alphabeta T cell receptor. *J Mol Med (Berl)* 92: 735-741.
3. Thompson EC (2012) Focus issue: Structure and function of lymphoid tissues. *Trends Immunol* 33: 255.
4. Luckheeram RV, Zhou R, Verma AD, et al. (2012) CD4⁺ T cells: differentiation and functions. *Clin Dev Immunol* 2012: 925135.
5. Zhu J, Yamane H, Paul WE (2010) Differentiation of effector CD4 T cell populations (*). *Annu Rev Immunol* 28: 445-489.
6. Zhang N, Bevan MJ (2011) CD8⁺ T cells: foot soldiers of the immune system. *Immunity* 35: 161-168.

7. Grabie N, Delfs MW, Westrich JR, et al. (2003) IL-12 is required for differentiation of pathogenic CD8⁺ T cell effectors that cause myocarditis. *J Clin Invest* 111: 671-680.
8. Starbeck-Miller GR, Xue HH, Harty JT (2014) IL-12 and type I interferon prolong the division of activated CD8 T cells by maintaining high-affinity IL-2 signaling in vivo. *J Exp Med* 211: 105-120.
9. Anastas JN, Moon RT (2013) WNT signaling pathways as therapeutic targets in cancer. *Nat Rev Cancer* 13: 11-26.
10. Moon RT, Gough NR (2016) Beyond canonical: The Wnt and beta-catenin story. *Sci Signal* 9: eg5.
11. Moon RT, Kohn AD, De Ferrari GV, et al. (2004) WNT and beta-catenin signaling: diseases and therapies. *Nat Rev Genet* 5: 691-701.
12. Klaus A, Birchmeier W (2008) Wnt signaling and its impact on development and cancer. *Nat Rev Cancer* 8: 387-398.
13. Clevers H, Loh KM, Nusse R (2014) Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science* 346: 1248012.
14. van de Wetering M, de Lau W, Clevers H (2002) WNT signaling and lymphocyte development. *Cell* 109 Suppl: S13-19.
15. Pongracz J, Hare K, Harman B, et al. (2003) Thymic epithelial cells provide WNT signals to developing thymocytes. *Eur J Immunol* 33: 1949-1956.
16. Brunk F, Augustin I, Meister M, et al. (2015) Thymic Epithelial Cells Are a Nonredundant Source of Wnt Ligands for Thymus Development. *J Immunol* 195: 5261-5271.
17. Staal FJ, Meeldijk J, Moerer P, et al. (2001) Wnt signaling is required for thymocyte development and activates Tcf-1 mediated transcription. *Eur J Immunol* 31: 285-293.
18. Gounari F, Aifantis I, Khazaie K, et al. (2001) Somatic activation of beta-catenin bypasses pre-TCR signaling and TCR selection in thymocyte development. *Nat Immunol* 2: 863-869.
19. Weerkamp F, Baert MR, Naber BA, et al. (2006) Wnt signaling in the thymus is regulated by differential expression of intracellular signaling molecules. *Proc Natl Acad Sci U S A* 103: 3322-3326.
20. Xu Y, Banerjee D, Huelsken J, et al. (2003) Deletion of beta-catenin impairs T cell development. *Nat Immunol* 4: 1177-1182.
21. Mulroy T, Xu Y, Sen JM (2003) beta-Catenin expression enhances generation of mature thymocytes. *Int Immunol* 15: 1485-1494.
22. Wu B, Crampton SP, Hughes CC (2007) Wnt signaling induces matrix metalloproteinase expression and regulates T cell transmigration. *Immunity* 26: 227-239.
23. Willinger T, Freeman T, Herbert M, et al. (2006) Human naive CD8 T cells down-regulate expression of the WNT pathway transcription factors lymphoid enhancer binding factor 1 and transcription factor 7 (T cell factor-1) following antigen encounter in vitro and in vivo. *J Immunol* 176: 1439-1446.

24. Alvarez-zavala M, Aguilar-lemarroy A, Jave-suarez LF (2015) WNT7a as a new feature of the T mature cells; expression of the WNT7a diminish in a highly activated and proliferative T cells after TCR activation and IL2 stimulus while canonical targets of WNT signaling pathway are overexpress. *Front Immunol* 6.
25. Driessens G, Zheng Y, Locke F, et al. (2011) Beta-catenin inhibits T cell activation by selective interference with linker for activation of T cells-phospholipase C-gamma1 phosphorylation. *J Immunol* 186: 784-790.
26. van Loosdregt J, Fleskens V, Tiemessen MM, et al. (2013) Canonical Wnt signaling negatively modulates regulatory T cell function. *Immunity* 39: 298-310.
27. Keerthivasan S, Aghajani K, Dose M, et al. (2014) beta-Catenin promotes colitis and colon cancer through imprinting of proinflammatory properties in T cells. *Sci Transl Med* 6: 225ra228.
28. Yu Q, Sharma A, Oh SY, et al. (2009) T cell factor 1 initiates the T helper type 2 fate by inducing the transcription factor GATA-3 and repressing interferon-gamma. *Nat Immunol* 10: 992-999.
29. Notani D, Gottimukkala KP, Jayani RS, et al. (2010) Global regulator SATB1 recruits beta-catenin and regulates T(H)2 differentiation in Wnt-dependent manner. *PLoS Biol* 8: e1000296.
30. Prlic M, Bevan MJ (2011) Cutting edge: beta-catenin is dispensable for T cell effector differentiation, memory formation, and recall responses. *J Immunol* 187: 1542-1546.
31. Ding Y, Shen S, Lino AC, et al. (2008) Beta-catenin stabilization extends regulatory T cell survival and induces anergy in nonregulatory T cells. *Nat Med* 14: 162-169.
32. Muranski P, Borman ZA, Kerkar SP, et al. (2011) Th17 cells are long lived and retain a stem cell-like molecular signature. *Immunity* 35: 972-985.
33. Dai W, Liu F, Li C, et al. (2016) Blockade of Wnt/beta-Catenin Pathway Aggravated Silica-Induced Lung Inflammation through Tregs Regulation on Th Immune Responses. *Mediators Inflamm* 2016: 6235614.
34. Lee YS, Lee KA, Yoon HB, et al. (2012) The Wnt inhibitor secreted Frizzled-Related Protein 1 (sFRP1) promotes human Th17 differentiation. *Eur J Immunol* 42: 2564-2573.
35. Gattinoni L, Zhong XS, Palmer DC, et al. (2009) Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. *Nat Med* 15: 808-813.
36. Jeannet G, Boudousquie C, Gardiol N, et al. (2010) Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory. *Proc Natl Acad Sci U S A* 107: 9777-9782.
37. Zhao DM, Yu S, Zhou X, et al. (2010) Constitutive activation of Wnt signaling favors generation of memory CD8 T cells. *J Immunol* 184: 1191-1199.
38. Driessens G, Zheng Y, Gajewski TF (2010) Beta-catenin does not regulate memory T cell phenotype. *Nat Med* 16: 513-514; author reply 514-515.

39. Boudousquie C, Danilo M, Pousse L, et al. (2014) Differences in the transduction of canonical Wnt signals demarcate effector and memory CD8 T cells with distinct recall proliferation capacity. *J Immunol* 193: 2784-2791.
40. Richards MH, Narasipura SD, Seaton MS, et al. (2016) Migration of CD8+ T Cells into the Central Nervous System Gives Rise to Highly Potent Anti-HIV CD4dimCD8bright T Cells in a Wnt Signaling-Dependent Manner. *J Immunol* 196: 317-327.
41. Taghon T, Yui MA, Pant R, et al. (2006) Developmental and molecular characterization of emerging beta- and gammadelta-selected pre-T cells in the adult mouse thymus. *Immunity* 24: 53-64.
42. Wendorff AA, Koch U, Wunderlich FT, et al. (2010) Hes1 is a critical but context-dependent mediator of canonical Notch signaling in lymphocyte development and transformation. *Immunity* 33: 671-684.
43. Koch U, Fiorini E, Benedito R, 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.
44. Anderson G, Pongracz J, Parnell S, et al. (2001) Notch ligand-bearing thymic epithelial cells initiate and sustain Notch signaling in thymocytes independently of T cell receptor signaling. *Eur J Immunol* 31: 3349-3354.
45. Van de Walle I, De Smet G, Gartner M, et al. (2011) Jagged2 acts as a Delta-like Notch ligand during early hematopoietic cell fate decisions. *Blood* 117: 4449-4459.
46. Jaleco AC, Neves H, Hooijberg E, et al. (2001) Differential effects of Notch ligands Delta-1 and Jagged-1 in human lymphoid differentiation. *J Exp Med* 194: 991-1002.
47. Minter LM, Turley DM, Das P, et al. (2005) Inhibitors of gamma-secretase block in vivo and in vitro T helper type 1 polarization by preventing Notch upregulation of Tbx21. *Nat Immunol* 6: 680-688.
48. Jurynczyk M, Jurewicz A, Raine CS, et al. (2008) Notch3 inhibition in myelin-reactive T cells down-regulates protein kinase C theta and attenuates experimental autoimmune encephalomyelitis. *J Immunol* 180: 2634-2640.
49. Auderset F, Schuster S, Coutaz M, et al. (2012) Redundant Notch1 and Notch2 signaling is necessary for IFNgamma secretion by T helper 1 cells during infection with *Leishmania major*. *PLoS Pathog* 8: e1002560.
50. 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.
51. Elyaman W, Bassil R, Bradshaw EM, et al. (2012) Notch receptors and Smad3 signaling cooperate in the induction of interleukin-9-producing T cells. *Immunity* 36: 623-634.
52. Bailis W, Yashiro-Ohtani Y, Fang TC, et al. (2013) Notch simultaneously orchestrates multiple helper T cell programs independently of cytokine signals. *Immunity* 39: 148-159.

53. Anastasi E, Campese AF, Bellavia D, et al. (2003) Expression of activated Notch3 in transgenic mice enhances generation of T regulatory cells and protects against experimental autoimmune diabetes. *J Immunol* 171: 4504-4511.
54. Campese AF, Grazioli P, Colantoni S, et al. (2009) Notch3 and pTalpha/pre-TCR sustain the in vivo function of naturally occurring regulatory T cells. *Int Immunol* 21: 727-743.
55. Barbarulo A, Grazioli P, Campese AF, et al. (2011) Notch3 and canonical NF-kappaB signaling pathways cooperatively regulate Foxp3 transcription. *J Immunol* 186: 6199-6206.
56. Charbonnier LM, Wang S, Georgiev P, et al. (2015) Control of peripheral tolerance by regulatory T cell-intrinsic Notch signaling. *Nat Immunol* 16: 1162-1173.
57. Hue S, Kared H, Mehwish Y, et al. (2012) Notch activation on effector T cells increases their sensitivity to Treg cell-mediated suppression through upregulation of TGF-betaRII expression. *Eur J Immunol* 42: 1796-1803.
58. Wong KK, Carpenter MJ, Young LL, 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.
59. Riella LV, Ueno T, Batal I, 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.
60. Maekawa Y, Minato Y, Ishifune C, et al. (2008) Notch2 integrates signaling by the transcription factors RBP-J and CREB1 to promote T cell cytotoxicity. *Nat Immunol* 9: 1140-1147.
61. Sugimoto K, Maekawa Y, Kitamura A, et al. (2010) Notch2 signaling is required for potent antitumor immunity in vivo. *J Immunol* 184: 4673-4678.
62. Sierra RA, Thevenot P, Raber PL, et al. (2014) Rescue of notch-1 signaling in antigen-specific CD8+ T cells overcomes tumor-induced T-cell suppression and enhances immunotherapy in cancer. *Cancer Immunol Res* 2: 800-811.
63. Backer RA, Helbig C, Gentek R, et al. (2014) A central role for Notch in effector CD8(+) T cell differentiation. *Nat Immunol* 15: 1143-1151.
64. Mathieu M, Duval F, Daudelin JF, et al. (2015) The Notch signaling pathway controls short-lived effector CD8+ T cell differentiation but is dispensable for memory generation. *J Immunol* 194: 5654-5662.
65. Briscoe J, Therond PP (2013) The mechanisms of Hedgehog signaling and its roles in development and disease. *Nat Rev Mol Cell Biol* 14: 416-429.
66. Ingham PW, Nakano Y, Seger C (2011) Mechanisms and functions of Hedgehog signaling across the metazoa. *Nat Rev Genet* 12: 393-406.
67. Varjosalo M, Taipale J (2008) Hedgehog: functions and mechanisms. *Genes Dev* 22: 2454-2472.
68. Outram SV, Varas A, Pepicelli CV, et al. (2000) Hedgehog signaling regulates differentiation from double-negative to double-positive thymocyte. *Immunity* 13: 187-197.

69. Shah DK, Hager-Theodorides AL, Outram SV, et al. (2004) Reduced thymocyte development in sonic hedgehog knockout embryos. *J Immunol* 172: 2296-2306.
70. El Andaloussi A, Graves S, Meng F, et al. (2006) Hedgehog signaling controls thymocyte progenitor homeostasis and differentiation in the thymus. *Nat Immunol* 7: 418-426.
71. Sacedon R, Varas A, Hernandez-Lopez C, et al. (2003) Expression of hedgehog proteins in the human thymus. *J Histochem Cytochem* 51: 1557-1566.
72. Hager-Theodorides AL, Dessens JT, Outram SV, et al. (2005) The transcription factor Gli3 regulates differentiation of fetal CD4- CD8- double-negative thymocytes. *Blood* 106: 1296-1304.
73. Stewart GA, Lowrey JA, Wakelin SJ, et al. (2002) Sonic hedgehog signaling modulates activation of and cytokine production by human peripheral CD4+ T cells. *J Immunol* 169: 5451-5457.
74. Lowrey JA, Stewart GA, Lindey S, et al. (2002) Sonic hedgehog promotes cell cycle progression in activated peripheral CD4(+) T lymphocytes. *J Immunol* 169: 1869-1875.
75. Rowbotham NJ, Hager-Theodorides AL, Cebecauer M, et al. (2007) Activation of the Hedgehog signaling pathway in T-lineage cells inhibits TCR repertoire selection in the thymus and peripheral T-cell activation. *Blood* 109: 3757-3766.
76. Furmanski AL, Barbarulo A, Solanki A, et al. (2015) The transcriptional activator Gli2 modulates T-cell receptor signaling through attenuation of AP-1 and NFkappaB activity. *J Cell Sci* 128: 2085-2095.
77. Michel KD, Uhmman A, Dressel R, et al. (2013) The hedgehog receptor patched1 in T cells is dispensable for adaptive immunity in mice. *PLoS One* 8: e61034.
78. de la Roche M, Ritter AT, Angus KL, et al. (2013) Hedgehog signaling controls T cell killing at the immunological synapse. *Science* 342: 1247-1250.
79. Ye H, Zhang J, Wang J, et al. (2015) CD4 T-cell transcriptome analysis reveals aberrant regulation of STAT3 and Wnt signaling pathways in rheumatoid arthritis: evidence from a case-control study. *Arthritis Res Ther* 17: 76.
80. Weng AP, Ferrando AA, Lee W, et al. (2004) Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* 306: 269-271.
81. Tosello V, Ferrando AA (2013) The NOTCH signaling pathway: role in the pathogenesis of T-cell acute lymphoblastic leukemia and implication for therapy. *Ther Adv Hematol* 4: 199-210.
82. Jiao Z, Wang W, Hua S, et al. (2014) Blockade of Notch signaling ameliorates murine collagen-induced arthritis via suppressing Th1 and Th17 cell responses. *Am J Pathol* 184: 1085-1093.
83. Kijima M, Iwata A, Maekawa Y, et al. (2009) Jagged1 suppresses collagen-induced arthritis by indirectly providing a negative signal in CD8+ T cells. *J Immunol* 182: 3566-3572.
84. Sodsai P, Hirankarn N, Avihingsanon Y, et al. (2008) Defects in Notch1 upregulation upon activation of T Cells from patients with systemic lupus erythematosus are related to lupus disease activity. *Lupus* 17: 645-653.

85. Rauen T, Grammatikos AP, Hedrich CM, et al. (2012) cAMP-responsive element modulator alpha (CREMalpha) contributes to decreased Notch-1 expression in T cells from patients with active systemic lupus erythematosus (SLE). *J Biol Chem* 287: 42525-42532.
86. Bassil R, Zhu B, Lahoud Y, et al. (2011) Notch ligand delta-like 4 blockade alleviates experimental autoimmune encephalomyelitis by promoting regulatory T cell development. *J Immunol* 187: 2322-2328.
87. Dagklis A, Pauwels D, Lahortiga I, et al. (2015) Hedgehog pathway mutations in T-cell acute lymphoblastic leukemia. *Haematologica* 100: e102-105.
88. Gonzalez-Gugel E, Villa-Morales M, Santos J, et al. (2013) Down-regulation of specific miRNAs enhances the expression of the gene Smoothed and contributes to T-cell lymphoblastic lymphoma development. *Carcinogenesis* 34: 902-908.



AIMS Press

© 2016 Luokun Xie et al., licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>)