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Mini-Review

Notch system in the linkage of innate and adaptive immunity

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ABSTRACT

The lung is one of the most immunologically challenged organs and can be affected by a number of pathogens, including bacteria, virus, fungi, and parasites. The development and chronicity of pulmonary infection are determined by the early innate response to the pathogenic stimuli and are regulated at multiple levels. Initial studies have indicated that the interaction of Notch and Notch ligands plays a critical role during development, and further, the Notch system is an important bridge between APCs and T cell communication circuits. APCs are essential regulators of the innate immune response. They can respond to PAMPs through PRRs, which function in the recognition of pathogenic components and play an important role in the innate and adaptive immune response. T cells are essential regulators of adaptive immune responses and infectious diseases. However, the role of the Notch system in the cross-talk between APC and T cells during pulmonary infection is still poorly understood. In the present review, we discuss recent findings that explore the mechanisms underlying the role of Notch signaling in the linkage of innate and adaptive immunity, including pulmonary infection though PPRs and Notch activation. ***J. Leukoc. Biol.*** **92: 59–65; 2012.**

Introduction

The induction of immune responses during infection is initially sensed by the host's innate immune system and triggers a rapid, antiinfectious response that involves the release of proinflammatory cytokines and eventually leads to the activation of the adaptive immune response [1]. The first line of defense is initiated when cellular PRRs recognize PAMPs [2, 3]. Recognition of PAMPs by PRRs rapidly triggers an array of antimicrobial immune responses through the induction of various inflammatory cytokines, chemokines, and type I IFNs [1, 4]. Several families of PRRs, including TLRs, RIG-I-like receptors, nucleotide-binding oligomerization-like receptors, and DNA receptors (cytosolic sensors for DNA), are known to play a crucial role in host defense [5].

APCs, DCs, and macrophages can respond to pathogens through PRRs, which function in the recognition of infectious components and play an important role in the innate and adaptive immune responses [6, 7]. The innate immune response by APCs is initiated quickly to protect from overwhelming infectious organisms but with time, can also activate the adaptive immune response to the invading pathogens [8]. T cell differentiation is the hallmark of the initiation of the adaptive immune response [9]. Recent data have indicated that the controlled expression of Notch receptor proteins on T cells is essential for normal T cell development and maturation [10]. The connection between these PRRs and Notch pathways has helped to define the complex role of APCs in the regulation of T cell differentiation [11, 12]. We here review recent advances concerning the role of Notch signaling in T cell differentiation during infection and present our recent findings focusing on Notch functions in infectious models, which include mycobacterium antigen and influenza virus.

NOTCH SYSTEM IN T CELLS

Notch is a receptor system, which was originally shown to be involved in cell differentiation and survival [13]. Notch signaling is initiated by the ligand engagement of the Notch receptor. There are five mammalian ligands (Dll1, -3, and -4 and Jagged-1 and -2), each of which can promiscuously activate any of the four Notch receptors (Notch1, -2, -3, and -4) [12, 14]. Upon binding by Dll or Jagged ligands, Notch undergoes proteolytic cleavage catalyzed by a disintegrin and metalloproteinase proteases and the GSI, leading to the translocation of the N-ICD into the nucleus. N-ICD interacts with the transcrip-

Abbreviations: Dll=Delta-like, DNMAML=dominant-negative mastermind allele, EAE=experimental autoimmune encephalomyelitis, Eomes=eomesodermin, Foxp3=forkhead box p3, GSI=γ-secretase inhibitor complex, HS=hypersensitive, MAML=mastermind allele, N-ICD=Notch intracellular domain, RBP-J=recombination signal-binding protein for Ig-κJ region, RIG-I=retinoic acid-induced gene-I, RORγt =retinoic acid receptor-related orphan receptor γt, RSV=respiratory syncytial virus, Tbx=T-box transcription factor, Teff=effector T cell, Treg=regulatory T cell

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tional repressor, RBP-J. The N-ICD interaction with RBP-J displaces transcriptional corepressors from RBP-J and also recruits MAML protein. The new transcriptional complex of N-ICD-RBP-J-MAML converts RBP-J from a repressor to a transcriptional activator [12, 14–16].

In the last decade, it has been demonstrated that Notch signaling pathways contribute to the hematopoietic and immune systems, including roles in the development of embryonic hematopoietic stem cells and in multiple lineage decisions of developing lymphoid and myeloid cells [17]. Notch signaling during lymphoid development has been studied extensively, and its role in determining cell fate at many stages during T cell development is well characterized [17]. Notch-mediated responses have been shown to be involved in T cell lineage maturation from double-negative pro-T cells to double-positive $CD4^+/CD8^+$ T cells in the thymus [18–20]. Studies have mapped out this latter mechanism in the thymus, which helps to explain how T cells develop through the early differentiation stages [21–25]. Other studies have shown that Notch signaling is also involved in T cell differentiation into mature $CD4^+$ and $CD8^+$ T cells [26, 27]. Mature $CD4^+$ T cells are essential regulators of adaptive immune responses and inflammatory diseases. Upon antigenic stimulation by APCs through MHC class II proteins, naive $CD4^+$ T cells become activated, expand, and differentiate into various Teff helper subsets, termed Th1, Th2, Th17, and Tregs, which are each characterized by the production of distinct cytokines and effector functions [17]. On the other hand, $CD8^+$ T cells, responding to specific peptide-MHC complexes through MHC class I proteins, differentiate into CTLs and memory cells [17]. **Table 1** summarizes and provides references for some of the recent studies regarding the role of Notch in T cell immune responses. For a more extensive overview of Notch signaling, we refer to refs. [13, 44, 45].

NOTCH IN Th1 IMMUNE RESPONSE

Th1 cells normally produce the proinflammatory cytokine IFN-γ to stimulate the clearance of intracellular infections induced by microbial and viral infections, whereas an excessive Th1 response can cause autoimmunity [46]. A number of studies have demonstrated that Notch proteins are important in the induction of Th1 responses. In the presence of functional MyD88, PAMP binding to TLR up-regulates Dll4, which causes the differentiation of naïve Th cells to a Th1 phenotype [21, 28, 29, 47]. Furthermore, when Dll ligands are overexpressed on APC or are cross-linked as fusion proteins, they also promote Th1 cell differentiation [22]. Th1 cell responses were reduced in vivo following the administration of blocking Dll-Fc fusion proteins or of a neutralizing antibody, which was specific for Dll1 [22, 30, 31]. However, the functional difference in Th1 cell differentiation, driven by Dll1 and Dll4, is still poorly understood. Notch1 can regulate Tbx21 directly through complexes formed on the Tbx21 promoter (of the T-bet gene), which plays an important role in Th1 cell differentiation and IFN-γ production [32]. Also, in this study, ectopic expression of activated Notch1 restored Tbx21 transcription and IFN-γ production in Th1 cells that had the Notch pathway inhibited by the use of GSI inhibitors [32]. In contrast, another study in Notch signal-deficient mice demonstrated a normal Th1 cell response, thus questioning the role of endogenous Notch in normal Th1 cell differentiation [25]. Reconciliation of these diverging results will require further studies. Another factor affecting Th1 differentiation is IL-12, as naïve Th cells are skewed to the Th1 phenotype in the presence of exogeneously added IL-12 [48]. When IL-12 is absent or present at suboptimal levels, notch Dll ligands can positively affect Th1 cell differentiation [31]. However, genetic inactivation of RBP-J or Notch1 and Notch2 had no effect on Th1 cell differentiation [17, 21, 25, 26], suggesting that Notch signaling is not essential but has an accessory role in Th1 cell differentiation.

NOTCH IN Th2 IMMUNE RESPONSE

Not only is IL-4 important for the differentiation of Th2 cells, but once differentiated, these Th2 cells themselves produce IL-4, which stimulates the clearance of parasite infections [46]. In contrast, an excessive Th2 response can lead to allergic responses. Notch signaling has also been implicated in Th2 cell differentiation. In fact, genetic loss of T cell function studies have shown that Notch is essential for Th2 cell responses under various physiologically relevant conditions, including parasite infections [21, 25, 26]. In contrast to Th1 cell differentiation, the differentiation of naïve Th cells to a Th2 phenotype occurred in the absence of functional MyD88, when Jagged was constitutively expressed [21, 30]. In particular, the expression of Jagged is induced in DCs in response to Th2-skewed conditions, including parasites, allergens, or proinflammatory mediators, such as PGE2 [21, 36, 49]. Th2 cell responses are severely diminished in the absence of T cell-specific expression of Notch receptors 1 and 2 and pan-Notch inhibition mediated by RBP-J knockout or expression of a DNMAML [25, 26, 50], suggesting that Th2 cell differentiation by the Notch pathway is dependent on RBP-J. This requirement for RBP-J expression in Th2 cell differentiation stands in contrast to its accessory role in Th1 differentiation as mentioned above. Recent studies indicated that Notch promotes activity of the upstream GATA3 promoter, resulting in transcription of the GATA3 gene, a necessary transcription factor for Th2 cell differentiation [21]. Fang et al. [27] demonstrated that Notch regulates GATA3 expression by augmenting amounts of the alternative exon 1a transcript in $CD4^+$ T cells. Also, GATA3 might help to render the IL-4 enhancer, DNase I HS (HS5) accessible to Notch, which then results in increased IL-4 gene expression and protein levels [21, 27]. Furthermore, Notch directly regulates transcription of the IL-4 gene by binding to the HS5 enhancer [11]. Taken together, these data provide two molecular mechanisms by which Notch signaling can promote Th2 cell differentiation, namely, GATA3-dependent and GATA3-independent pathways.

NOTCH IN Th17 IMMUNE RESPONSE

In addition to the well-described Th1 and Th2 cell subsets, IL-17-producing T cells have recently been described and

TABLE 1. Summary of References for Recent Studies Regarding the Role of Notch in T Cell Immune Responses

	APC	Notch ligand	Notch receptor	In vivo model	Usage	Reference
Th1	DC (BM)	Dll4		No	LPS treatment	[21]
	plate-bound Dll1-Fc	Dll1	Notch 3	Yes (*Leishmania major*)	DII1-Fc injection into *L. major*-infected mice	[22]
	DC (BM)	Dll4		Yes (RSV)	MyD88-deficient mice	[28]
	Dll1 or Dll4 retrovirus-transduced IL-12$^{-/-}$ DCs	Dll1 Dll4		No	Forced expression of Dll	[29]
	Jagged1-Fc and Delta1-Fc-fusion^2 proteins	Dll1 Jagged1		Yes (EAE)	In vivo blockade of Jagged1 and Dll1	[30]
	CD8$^-$ DCs (spleen)	Dll4		No	LPS treatment	[31]
			Notch 1	Yes (EAE)	GSI inhibitor treatment	[32]
			Notch 3	Yes (EAE)	In vivo blockade of Notch 3	[33]
	Macrophage	Dll1		Yes (influenza virus)	In vivo blockade of Dll1	[34]
	DC (BM)	Dll4		Yes (RSV)	In vivo blockade of Dll4	[35]
Th2	DC (BM)	Jagged1	Notch 1	No	LPS treatment	[21]
				Yes (*L. major*)	Mice that lack Notch signaling in CD4$^+$ T cells	[25]
			Notch 1 Notch 2	No	RBP-J-deficient (RBP$^{fl/-}$-CD4Cre) mice	[26]
			Notch 1	No	ROSA26-DNMAML mice with CD4-cre mice	[27]
	DC (BM)	Jagged2		No	Immunization of *Schistosoma mansoni* egg antigen	[36]
Th17	DC (BM)	Dll4		Yes (*Mycobacterial granuloma*)	TLR9-deficient mice and in vivo blockade of Dll4	[37]
			Notch 3	Yes (EAE)	In vivo blockade of Notch 3	[33]
Treg		Dll1 Dll4 Jagged1	Notch 1	Yes (asthma)	CD4$^+$ T cells expressing Foxp3 and membrane-bound TGF-β	[23]
			Notch 1	Yes (autoimmune hepatitis)	Notch1 antisense mice	[38]
	Lin$^-$Sca-1$^+$c-kit$^+$ hematopoietic progenitor cells	Jagged2	Notch 3	Yes (autoimmune diabetes)	Adoptive transfer of hematopoietic progenitor cells into NOD mice	[39]
CD8$^+$ T	DC (BM) infected with a retrovirus encoding Dll1	Dll1	Notch 2	No	Notch2$^{f/f}$E8I-Cre$^+$ mice	[40]
			Notch 1	No	Notch1 antisense mice	[41]
		Dll1	Notch 1	Yes (asthma)	Administration of Dll1-Fc	[42]
	Macrophage	Dll1		Yes (influenza virus)	In vivo blockade of Dll1	[34]
	DC (BM)	Dll4		Yes (RSV)	In vivo blockade of Dll4	[35]
$\gamma\delta$T cells				No	Hes-1-deficient mice	[43]

BM, Bone marrow; NOD, nucleotide-binding oligomerization.

named Th17 cells [51, 52]. DC-derived Notch ligand Dll4 regulates the differentiation of naïveTh cells into Th17 cells through a MyD88-dependent pathway, in response to *Mycobacterium* antigens [37], and also up-regulates Th17 cell-specific transcription factor RORγt [53]. A recent study revealed a direct connection between Dll4-induced RBP-J and RORγt through a consensus RBP-J-binding site on the RORγt promoter, similar to Th2 differentiation [53]. In an EAE model, pathogenic Th1 and Th17 cells develop in the CNS, causing autoimmunity. GSI-mediated inhibition of Notch signaling in this disease model resulted in reduced Th1 and Th17 cytokines [32, 33]. In addition, specific antibodies against Dll1,

which attenuated EAE, had the opposite effect, caused by antibodies against Jagged1, which exacerbated EAE [30]. These results suggest that Dll ligands on DCs seem to be involved in the promotion of pathogenic Th1 and Th17 cells, whereas Jagged ligands might suppress autoimmunity [17].

NOTCH IN Treg IMMUNE RESPONSE

Tregs, which specifically express the forkhead family transcription factor Foxp3, are essential for the maintenance of immunological self-tolerance and immune homeostasis [54]. TGF-β is a key cytokine that induces Foxp3 expression and a regulatory phenotype in peripheral T cells (Tregs) [54]. Several reports showed that Notch signaling can enhance Treg differentiation and function in vitro [17, 55]. For example, Samon et al. [38] demonstrated that Notch1 and TGF-β signaling pathways cooperatively regulate Foxp3 expression and Treg maintenance in vitro and in vivo. Another report showed that exposure of Tregs to Jagged2-expressing hematopoietic progenitor cells resulted in Treg proliferation and prevented the development of diabetes in an experimental autoimmune disease model in mice [17, 39]. Collectively, these results indicate that Notch signaling has a critical role in sustaining Foxp3 expression in Tregs to maintain their immune-suppressive function. Thus, Notch signaling has been linked to many aspects of peripheral T cell immune responses, as well as to T cell development. For some of these functions, Notch may not be absolutely required. However, Notch signaling does appear to be critical in supporting immune responses. There is clearly more work needed, especially in in vivo models, to understand the full spectrum of Notch functions in the linkage between innate and adoptive immunity.

NOTCH IN OTHER T CELL IMMUNE RESPONSE

The $CD8^+$ T cell is also an essential component of the adaptive immune response to many pathogens [56]. Upon engagement with antigens, naïve $CD8^+$ T cells rapidly expand and differentiate into CTLs, producing cytokines, such as IFN-γ and the effector molecules, perforin and granzyme B [57]. Tbx—T-bet and Eomes—are important inducers of genes involved in the acquisition of CTL function and in the responsiveness to cytokines that regulate the survival of long-lived memory T cells [58, 59]. Recent data demonstrated that signaling mediated by Dll1 and Notch2 was required for full cytotoxic activity of CTLs and that Notch2 directly controlled transcription of the gene encoding granzyme B, which is independent of Eomes [40]. Another study indicated that Notch1 regulates the expression of Eomes, perforin, and granzyme B through N-ICD binding to the promoters of these effector molecules [41]. In an in vivo allergic asthma model, Notch1 is expressed on memory $CD8^+$ Teffs, and Dll1 expression is an effective inhibitor of allergic asthma responses [42]. However, the difference between Notch1 and Notch2 in the regulation of these effector molecules is not clearly understood.

Unlike conventional $\alpha\beta$ T cells, $\gamma\delta$ T cells constitute a whole system of functionally specialized subsets that are involved in the innate immune responses against tumors and pathogens and the regulation of immune responses, cell recruitment and activation, and tissue repair [60, 61]. In addition to Th17 cells, there is accumulating evidence that $\gamma\delta$ T cells could be the major source of IL-17 in various murine models of infection including *Mycobacterium tuberculosis*, *Escherichia coli*, and *Listeria monocytogenes* [62, 63]. Recent published data clearly demonstrated that Notch ligand Dll4 expression is correlated with the development of IL-17-producing $\gamma\delta$ T cells [43]. The importance of Notch signaling in IL-17-producing $\gamma\delta$ T cells in in vivo models remains unknown.

THE ROLE OF NOTCH PATHWAY FOR REGULATION OF BACTERIAL INFECTION

It has been estimated that one-third of the world's population is infected with *M. tuberculosis* and that tuberculosis is one of the most well-characterized, bacteria-induced granulomagenic diseases [64]. Whereas the mechanism of granuloma formation is not clear, this distinct cellular response is considered a histologic hallmark for a protective immune response, involving innate and adaptive immunity. Our group has studied Dll4, the primary Notch ligand, which is up-regulated by mycobacteial infection of DCs. When Dll4 was specifically blocked in vivo during mycobacteria-induced pulmonary granuloma formation, Th17 cellular responses were inhibited significantly, and larger granulomas were observed [37]. Moreover, in in vitro experiments, anti-Dll4 antibody specifically blocked IL-17 production by $CD4^+$ T cells, whereas overexpression of Dll4 augmented IL-17 production by $CD4^+$ T cells, suggesting that Dll4 plays an important role in promoting Th17 activity during a mycobacterial challenge [37] (**Fig. 1A**). These data suggest that an understanding of Dll4 regulation of Th17 responses through Notch may provide mechanistic approaches for modifying and controlling the immune response induced by the Th17 phenotype, including pathogenesis of not only bacterial and fungus infection but also autoimmune disease, allergic responses, and cancer.

THE ROLE OF NOTCH PATHWAY FOR REGULATION OF VIRAL INFECTION

Influenza viruses cause annual epidemics and occasional pandemics that have claimed the lives of millions [65]. Innate and acquired immunity is essential for protection against influenza virus, and it has been suggested that Notch and Notch ligands may provide a key bridge between these two arms of the immune system. Our recent data demonstrated that macrophages, but not DCs, increased Notch ligand Dll1 expression following influenza virus stimulation [34]. Dll1 expression on macrophages was dependent on the RIG-I-induced type-I IFN pathway and not on the TLR3-Toll-IL-1R domain-containing adaptor-inducing IFN-β pathway. IFN-$\alpha R^{-/-}$ mice failed to induce Dll1 expression on lung macrophages and had enhanced mortality during influenza virus infection. Further-

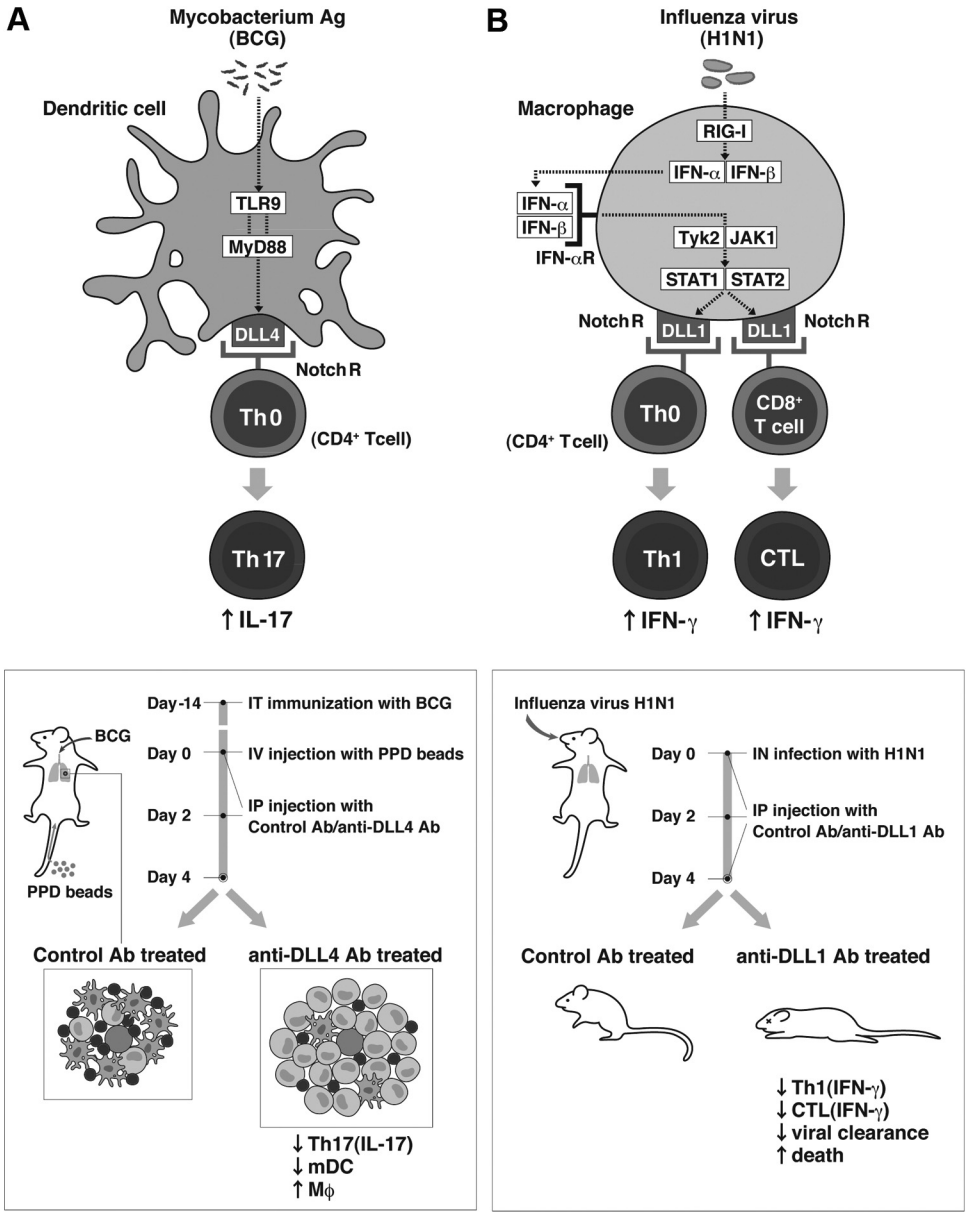

Figure 1. Schematic representation of the role of Notch ligands in our models. (A) The role of Notch ligand (Dll4) on a mycobacterium-induced granuloma model. In vivo granuloma formation induced by bacillus Calmette-Guérin (BCG)/mycobacterium antigen demonstrates larger granuloma formation in anti-Dll4 antibody-treated mice with decreased numbers of Th17 cells and mDCs and an increased number of macrophages (MΦ) in the lungs when compared with lung granulomas from mice treated with control antibody. The decreased expression of Dll4 led to the abrogation of the Th17 phenotype in the anti-Dll4 antibody-treated mice with a concomitant increase in granuloma size. The TLR9 signaling pathway, through MyD88 on DCs, plays a central role by up-regulating Dll4 and Th17-related cytokines, including IL-17, IL-6, IL-23(p19/p40), and TNF-α, in response to *Mycobacterium* antigens. IT, Intratracheal; IV, i.v.; IP, i.p.; PPD, mycobacteria-derived, purified protein derivative; mDC, myeloid DC. (B) The role of the Notch ligand (Dll1) on the influenza virus H1N1 infection model. Macrophages play an important role in regulating IFN-γ production from $CD4^+$ and $CD8^+$ T cells through a RIG-I-induced type-I IFN-dependent pathway (including the JAK-STAT pathway), which up-regulates the Notch ligand Dll1. Our in vivo influenza H1N1 infectious model demonstrates higher mortality and impaired viral clearance in anti-Dll1antibody-treated mice with decreased IFN-γ production when compared with control IgG-treated mice. IN, Intranasal.

more, specific neutralization of Dll1 during influenza virus challenge induced higher mortality, impaired viral clearance, and decreased levels of IFN-γ. Dll1 expression on macrophages specifically regulates IFN-γ levels from $CD4^+$ and $CD8^+$ T cells in vitro [34] (Fig. 1B). These data suggest that Notch signaling through macrophage-expressing Dll1 is critical in providing an antiviral response during influenza infection and provides a link between innate and acquired immunity. The Flavell group [21] has shown that Delta ligands on APCs promote Th1 differentiation, whereas the Yasutomo group [22] has demonstrated that Dll1 interaction with Notch3 on T cells promotes development toward the Th1 phenotype. Also, it has been shown that $CD8^-$ DCs can direct Th1 differentiation by an IL-12-independent and Dll4-dependent mechanism [31]. Thus, our work agrees with this notion that Dll ligands are important in driving Th1 cell differentiation. However, we are still investigating the type of Notch receptor involved in our model system.

Our colleagues have also studied the role of Notch signaling during RSV infection, which is a leading cause of bronchiolitis, pneumonia, and allergic asthma in young children worldwide. Notch ligand Dll4 was up-regulated on bone marrow-derived DCs after RSV infection through a MyD88-dependent pathway [28]. When specifically blocking Dll4 in vivo by passive immunization during RSV infection, a more intense pathogenic response was observed that included increases in airway hyperreactivity and mucus hypersecretion [35]. This response was characterized by elevated Th2 cytokine production, which could be reversed in vitro by culturing T cells under conditions of overexpression of Dll4 [35]. The different Notch ligand expression patterns caused by influenza virus and RSV might be a result of different PRRs. Whereas the main PRR

signaling pathway for influenza virus is a RIG-I-induced type-I IFN-dependent pathway in macrophages, which for RSV, is a TLR-induced, MyD88-dependent pathway in DCs. However, the specific mechanistic link governing the connection between each of the viral products and Notch signals remains unknown. We are now investigating the role of Notch signaling during herpes DNA virus infection.

CONCLUDING REMARKS

A number of recent studies clearly have demonstrated a critical role for Notch signaling in the regulation of APCs and T cells, an important connection between the innate and adaptive immune response. Whereas the relevance of APC, PRRs, and Notch in the activation of the mature immune system is a story in its infancy, the data to date demonstrate how the maturing immune system relies on a diverse set of molecules and cell populations to fine-tune the system for the most appropriate and least pathogenic responses. However, future studies need to include more relevant in vivo studies to confirm and expand the early findings of in vitro studies. One of our recent findings indicated that Notch ligand Dll4 caused an increase in the expansion of Th2 memory cells and a decrease in effector cell proliferation in a parasitic study model [66]. This study suggests that the Notch pathway also contributes to the different responses of memory and Teffs to Notch ligands, whereas it has been well established that Notch ligands can have opposing effects on T cell differentiation, depending on the immune environment [21]. More in-depth studies using mice that are deficient in each Notch and Notch ligand protein will provide data about how the different Notch ligands (Delta and Jagged ligands) control the different types of T cell responses during physiological conditions. Validation of the in vitro data requires a number of in vivo studies using diverse infectious disease models, which include bacteria, virus, parasites, and fungus, as well as autoimmune and allergy models. Moreover, the cellular constituents of the lung immune system of the lung are diverse and include not only leukocytes, such as macrophages, DCs, neutrophils, mast cells, and lymphocytes, but also, it is clear that epithelial cells and fibroblasts play critical roles in the lung defense system. Further knowledge of the regulation of the Notch system in these cells and contextual interactions between these populations may provide mechanistic approaches for modifying and controlling the immune response during infectious diseases in clinically relevant translational studies.

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KEY WORDS:
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