Mini Review

Notch system in influenza A/H1N1 virus infection

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Influenza viruses cause annual epidemics and occasional pandemics that have claimed the lives of millions. Both innate and acquired immunity are essential for protection against influenza virus. A viral infection is initially sensed by the host innate system, triggering a rapid antiviral response that involves the release of proinflammatory cytokines, and eventually leads to the activation of the adaptive immune response. The innate immune response is initiated when cellular pathogen recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs). The innate immune response by antigen presenting cells (APCs), including dendritic cells (DCs) and macrophages, is initiated quickly to protect from overwhelming infectious organisms, but with time, also can activate the adaptive immune response to the invading pathogens. The adaptive immune response is essential for purging a diverse repertoire of invading pathogens, and $CD4^+$ and $CD8^+$ T cells are required for successful eradication of pathogens. 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. However, the role of Notch system during influenza virus infection is still unknown. Here we review our recent study which shows that Notch signaling through macrophage-dependent Delta-like 1 (Dll1) is critical in providing an anti-viral response during influenza virus infection by linking innate and acquired immunity.

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Abbreviations: APC: antigen-presenting cell, DC: dendritic cell, Dll: Delta-like, GSI: γ-secretase inhibitor, PRR: pathogen recognition receptor, PAMP: pathogen-associated molecular pattern

Key words cytotoxic T lymphocyte, Notch ligand Delta-like 1, helper T cell, macrophage, viral immunity

A short overview of Notch system

Notch is a heterodimeric cell-surface receptor that is involved in a broad range of differentiation processes[1]. It is composed of an extracellular ligand-binding domain that is non-covalently associated with a single-pass transmembrane domain[1, 2]. The notch signaling pathway regulates many aspects of embryonic development, as well as differentiation processes and tissue homeostasis in multiple

adult organ systems[1]. There are two distinct families of Notch ligands in mammals, known as the Delta-like ligands (consisting of DLL1, DLL3, and DLL4) and the Jagged ligands (Jagged1 and Jagged2); both DLL and Jagged proteins trigger the canonical Notch signalling pathway[3]. In the canonical signalling pathway, binding of a ligand to Notch results in the cleavage of the receptor at a site in the transmembrane portion. This cleavage is mediated by a γ-secretase complex, and inhibitors of this complex are frequently used to experimentally inhibit Notch activation. Upon binding by either Dll or Jagged ligands, Notch undergoes proteolytic cleavage catalyzed by Adam proteases and the γ-secretase complex, leading to the translocation of the notch intracellular domain (N-ICD) into the nucleus. N-ICD interacts with the transcriptional repressor, recombination-signal-binding protein for immunoglobulin-κJ region (RBP-J). The N-ICD interaction with RBP-J displaces transcriptional co-repressors from RBP-J and also recruits Mastermind (MAML) protein. The new transcriptional complex of N-ICD-RBP-J-MAML converts RBP-J from a repressor to a transcriptional activator[3, 4]. Although unique functions for each ligand have been described, the underlying mechanisms for these differences are still unclear. Regulation of Notch signaling is associated with several human disorders, including cancer[5]. Recently, it has become evident that Notch signaling plays important roles within the hematopoietic and immune systems. In the mature immune system, the Notch pathway has been described as a signaling mechanism involved in regulating cell lineage choices for T cells[2, 6].

Notch pathway in the linkage of APC and T cell

Pathogens such as bacteria, helminths, fungi, and also viruses are recognized by antigen-presenting cells (APCs), which then activate $CD4^+$ T helper (Th) cells[7]. These cells drive adaptive immunity and induce specific responses against these microbes[8]. It has well studied that the different types of APC and their availability to display particular cytokine production profiles, pathogen recognition receptors (PRRs), and costimulatory molecules are key determinants for Th differentiation[9]. In addition, studies have demonstrated that Notch proteins are also important in the induction of Th responses[2, 6]. Th1 cells produce IFN-γ and are involved in the fight against intracellular pathogens[10], whereas Th2 cells secrete interleukin-4 (IL-4), IL-5, and IL-13 and provide immunity to helminthes[11]. For example, in the presence of functional Myeloid differentiation factor 88 (MyD88), a adaptor molecule of Toll-like receptor (TLR), pathogen-associated molecular pattern (PAMP) binding to TLR upregulates Dll1 or Dll4 on APCs, which causes the differentiation of naïve Th cells to a Th1 phenotype. On the other hand, the differentiation of naïve Th cells to a Th2 phenotype occurred in the absence of functional MyD88 when Jagged was constitutively expressed on APCs[2, 12]. Moreover, recent finding described IL-17-producing T cells, named Th17[13]. Some extracellular bacteria and fungi are cleared by the presence of Th17 cells that recruit neutrophils to the site of infection[14]. We have demonstrated that Dll4 induction on DCs can specifically promote the generation of Th17 cells in the pathogenesis of mycobacteria-elicited granulomatous immune response[15]. Notch signaling is also associated with the differentiation of naïve $CD8^+$ T cells to cytotoxic T lymphocytes (CTLs), a process that is in part mediated by the up-regulation of the transcriptional regulator eomesodermin (Eomes), which regulates the expression of perforin and granzyme B, 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[16]. Recent data demonstrated that signaling mediated by Dll1 was required for full cytotoxic activity of CTLs[17, 18]. However, how Dll- and Jagged-expressing APCs differ in their ability to induce Notch signaling is unclear and will need to be answered in the future.

Notch ligand Delta-like 1 (Dll1) up-regulation in influenza virus challenged macrophages

Many studies have shown that notch ligand regulation on APCs is a key event for T cell differentiation. Using BM-derived DCs and macrophages, we assessed the gene expression profile of Notch ligands on APCs following influenza virus stimulation. Of the five Notch ligands, Dll1 is the only Notch ligand specifically up-regulated on macrophages following influenza stimulation, but it is not expressed on DCs[19]. Also in murine influenza virus infectious model, the peak expression of Dll1 on lung macrophages in mice coincides with the period of peak inflammation after H1N1 infection. Among the cytokines induced during the innate immune response, activation of type-I IFNs is the most powerful defense mechanism against in-

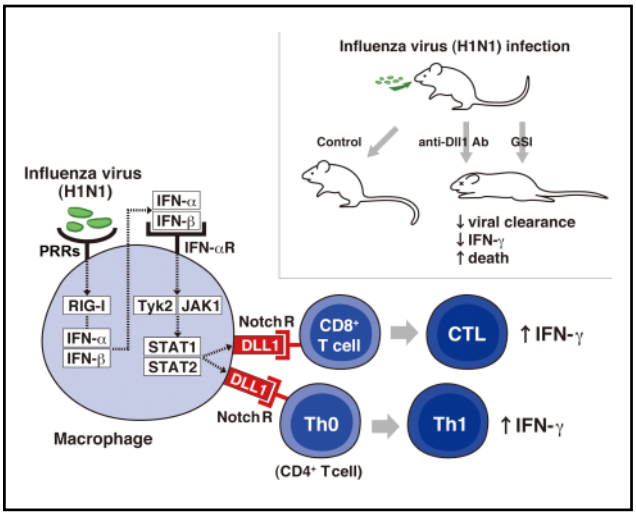

Fig.1 Schematic representation of the role of Notch ligand (Dll1) on influenza virus H1N1 infection model

Macrophages play an important role in regulating IFN-γ production from both $CD4^+$ and $CD8^+$ T cells through RIG-I-induced type-I IFN-dependent pathway (including JAK-STAT pathway) that upregulates the Notch ligand Dll1. Our *in vivo* influenza H1N1 infectious model demonstrates higher mortality and impaired viral clearance in anti-Dll1Ab treated or GSI treated mice with decreased IFN-γ production when compared with control treated mice.

fluenza viral replication and spread[20]. Moreover, macrophages, but not DCs, showed enhanced Notch ligand Dll1 expression in response to influenza virus and to type-I IFN cytokines, which suggested that Dll1 induction is dependent on type-I IFNs. We also confirmed this by showing that IFNαR$^{-/-}$-derived macrophages completely failed to induce Dll1. Influenza virus amplifies the type-I IFN response via a positive-feedback loop that activates JAK-1 and Tyk-2 kinases, which leads to the phosphorylation and dimerization of STAT1 and STAT2 protein[21, 22]. Our studies also showed impaired Dll1 induction on macrophages from STAT1$^{-/-}$ mice and macrophages treated with a JAK-1 inhibitor. PRRs that recognize influenza virus RNA, have been shown to be a key initiator of type-I IFN response in infected cells. These PRRs rely on the RIG-I-like signaling pathway, composed of RIG-I and MDA5. RIG-I-knocked down macrophages, not MDA-5-knocked down macrophages, expressed decreased Notch ligand Dll1 with significantly decreased type-I IFN cytokine production following influenza virus stimulation. Thus, Our results show that influenza virus-induced type-I IFNs are exclusively RIG-I dependent and that their production is essential for the induction of Dll1 through the IFNαR and the JAK-1/STAT1/2 signaling pathway[19] (Fig.1).

Role of DLL1-expressed macrophage against influenza virus infection

Macrophages are important components of innate host defense and can play a critical role in limiting the severity of influenza virus infection. We showed that specific neutralization of Dll1 during influenza virus challenge induced higher mortality, impaired viral clearance, and decreased levels of IFN-γ. In addition, treatment of Notch signaling by using γ-secretase inhibitor (GSI), a Notch signaling inhibitor, during influenza infection led to higher mortality, higher virus load, and an impaired production of IFN-γ in lungs (Fig.1). Our findings indicated that induction of Dll1 on macrophages in response to influenza virus specifically regulated IFN-γ production from $CD4^+$ and $CD8^+$ T cells both *in vivo* and *in vitro*[19] (Fig.1). Dll1 is required for optimal IFN-γ production in response to Ag as previously described[23]. Several studies support our results showing that IFN-γ plays an important role in recovery from influenza viral infection by helping to clear the virus[20, 24]. Thus, macrophages are indispensable for the protection against influenza virus by their enhancement of Dll1 expression levels and up-regulating IFN-γ level from T cells through Notch signaling pathway.

Closing remarks

Our studies have revealed a critical role of Notch signaling in infectious model using influenza virus. In this model Dll1 influences the development of anti-viral immunity, and may provide mechanistic approaches for modifying and controlling the immune response against influenza virus infection. New type of influenza vaccine to induce Dll1 on macrophages might be one of candidate. For example, intranasal pre-treatment of poly(I:C), a double-stranded RNA (dsRNA) ligand which could induce type-I IFN and increase Dll1 expression on macrophages[19], provided high level of protection against lethal challenge with influenza virus[25]. However, Notch system seems more complicated system, because Notch signaling pathways contribute to immune systems including a role in multiple lineage decisions of developing lymphoid and myeloid cells. A better understanding of the regulation of the notch system might contribute a novel therapeutic approach for influenza virus infection.

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References

1) Bray SJ: Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol. 2006; 7: 678-689.
2) Amsen D, Antov A, Flavell RA: The different faces of Notch in T-helper-cell differentiation. Nat Rev Immunol. 2009; 9: 116-124.
3) Ehebauer M, Hayward P, Arias AM: Notch, a universal arbiter of cell fate decisions. Science. 2006; 314: 1414-1415.
4) Dallman MJ, Champion B, Lamb JR: Notch signalling in the peripheral immune system. Novartis Found Symp. 2003; 252: 268-266, discussion 276-278.
5) D'Souza B, Miyamoto A, Weinmaster G: The many facets of Notch ligands. Oncogene. 2008; 27: 5148-5167.
6) Radtke F, Fasnacht N, Macdonald HR: Notch signaling in the immune system. Immunity. 2010; 32: 14-27.
7) Kumar H, Kawai T, Akira S: Pathogen recognition by the innate immune system. Int Rev Immunol. 2011; 30: 16-34.
8) Trinchieri G, Sher A: Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol. 2007; 7: 179-190.
9) Carballido JM, Carballido-Perrig N, Schwarzler C, Lametschwandtner G: Regulation of human T helper cell differentiation by antigen-presenting cells: the bee venom phospholipase A2 model. Chem Immunol Allergy. 2006; 91: 147-158.
10) Kapsenberg ML: Dendritic-cell control of pathogen-driven T-cell polarization. Nat Rev Immunol. 2003; 3: 984-993.
11) Ito T, Schaller M, Raymond T, Joshi AD, Coelho AL, Frantz FG, Carson WFt, Hogaboam CM, Lukacs NW, Standiford TJ, Phan SH, Chensue SW, Kunkel SL: Toll-like receptor 9 activation is a key mechanism for the maintenance of chronic lung inflammation. Am J Respir Crit Care Med. 2009; 180: 1227-1238.
12) Amsen D, Spilianakis CG, Flavell RA: How are T(H)1 and T(H)2 effector cells made? Curr Opin Immunol. 2009; 21: 153-160.
13) Korn T, Bettelli E, Oukka M, Kuchroo VK: IL-17 and Th17 Cells. Annu Rev Immunol. 2009; 27: 485-517.
14) Matsuzaki G, Umemura M: Interleukin-17 as an effector molecule of innate and acquired immunity against infections. Microbiol Immunol. 2007; 51: 1139-1147.
15) Ito T, Schaller M, Hogaboam CM, Standiford TJ, Sandor M, Lukacs NW, Chensue SW, Kunkel SL: TLR9 regulates the mycobacteria-elicited pulmonary granulomatous immune response in mice through DC-derived Notch ligand delta-like 4. J Clin Invest. 2009; 119: 33-46.
16) Intlekofer AM, Takemoto N, Wherry EJ, Longworth SA, Northrup JT, Palanivel VR, Mullen AC, Gasink CR, Kaech SM, Miller JD, Gapin L, Ryan K, Russ AP, Lindsten T, Orange JS, Goldrath AW, Ahmed R, Reiner SL: Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. Nat Immunol. 2005; 6: 1236-1244.
17) Cho OH, Shin HM, Miele L, Golde TE, Fauq A, Minter LM, Osborne BA: Notch regulates cytolytic effector function in CD8+ T cells. J Immunol. 2009; 182: 3380-3389.
18) Skokos D, Nussenzweig MC: CD8- DCs induce IL-12-independent Th1 differentiation through Delta 4 Notch-like ligand in response to bacterial LPS. J Exp Med. 2007; 204: 1525-1531.
19) Ito T, Allen RM, Carson WF, Schaller M, Cavassani KA, Hogaboam CM, Lukacs NW, Matsukawa A, Kunkel SL: The critical role of Notch ligand Delta-like 1 in the pathogenesis of influenza A virus (H1N1) infection. PLoS Pathog. 2011; 7: e1002341.
20) Karupiah G, Chen JH, Mahalingam S, Nathan CF, MacMicking JD: Rapid interferon gamma-dependent clearance of influenza A virus and protection from consolidating pneumonitis in nitric oxide synthase 2-deficient mice. J Exp Med. 1998; 188: 1541-1546.
21) Goodman AG, Zeng H, Proll SC, Peng X, Cilloniz C, Carter VS, Korth MJ, Tumpey TM, Katze MG: The alpha/beta interferon receptor provides protection against influenza virus replication but is dispensable for inflammatory response signaling. J Virol. 2010; 84: 2027-2037.
22) Schindler C, Plumlee C: Inteferons pen the JAK-STAT pathway. Semin Cell Dev Biol. 2008; 19: 311-318.
23) Maekawa Y, Tsukumo S, Chiba S, Hirai H, Hayashi Y, Okada H, Kishihara K, Yasutomo K: Delta1-Notch3 interactions bias the functional differentiation of acti-

vated CD4+ T cells. Immunity. 2003; 19: 549-559.
24) Khoufache K, LeBouder F, Morello E, Laurent F, Riffault S, Andrade-Gordon P, Boullier S, Rousset P, Vergnolle N, Riteau B: Protective role for protease-activated receptor-2 against influenza virus pathogenesis via an IFN-gamma-dependent pathway. J Immunol. 2009; 182: 7795-7802.
25) Wong JP, Christopher ME, Viswanathan S, Karpoff N, Dai X, Das D, Sun LQ, Wang M, Salazar AM: Activation of toll-like receptor signaling pathway for protection against influenza virus infection. Vaccine. 2009; 27: 3481-3483.